Bone Health and Body Composition of Children and Adolescents with Growth Hormone Deficiency

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Abstract

Childhood onset growth hormone deficiency (CO-GHD) may contribute to low bone mass and alterations to body composition. This thesis consists of a series of studies utilising dual-energy X-ray absorptiometry (DXA), peripheral quantitative computerized tomography (pQCT) and biochemical assessment of bone health and body composition of CO-GHD. In addition, metabolic profiles, glucose metabolism as well as quality of life have been studied in these subjects. Furthermore, an interventional study of weight bearing exercise (WBE) was performed to explore its role in influencing the bone health of children and adolescents with CO-GHD.

Chapter 1, relevant literature reviews explore: bone structure, growth, development and strength; GH/IGF-1 system and its actions; CO-GHD and its impacts during childhood and transition; and WBE and its mechanism and impacts on bone health. Chapter 2 presents the rationale and specific aims of this thesis.

Chapter 3, a retrospective multicentre review of management of young adults with CO-GHD in four paediatric centres in Scotland during transition. Medical records of 130 eligible CO-GHD adolescents (78 males), who attained final height between 2005-2013 were reviewed. Of the 130, 74/130(57%) had GH axis re-evaluation by stimulation tests /IGF-1 measurements. Of those, 61/74(82%) remained GHD with 51/74(69%) restarting adult rhGH. Predictors of persistent GHD included an organic hypothalamic-pituitary disorder and multiple pituitary hormone deficiencies (MPHD). Despite clinical guidelines, there was significant variation in the management of CO-GHD in young adulthood across Scotland.

Chapter 4, a cross-sectional control study of bone DXA measurements in (n=21) subjects with CO-GHD treated with rhGH and had attained final height from 2005 to 2013 in a single tertiary paediatric centre compared to (n= 21) heights /age matched healthy controls. By applying different models of DXA adjustment, our analysis revealed lower TB-BMC for bone area in males with CO-GHD and lower LS-BMAD SDS in females with CO-GHD compared to matched controls. In addition, subjects with CO-GHD had lower LM for height and higher FM for height compared to controls, and this was more pronounced in males than females (p=0.04). The time of onset and aetiology of CO-GHD have a larger influence on accrual of bone mass in these patients. These findings indicate that adolescents with CO-GHD have a low bone mass, despite prior long term rhGH replacement therapy.

In chapter 5, we investigated bone health of subjects with CO-GHD at time of initial evaluation and retesting at final height. A total of 25 children (first time assessment group) undergoing GH stimulation tests for investigation of short stature (naive GHD-15, normal-10), and 11adolescents with CO-GHD (retesting group) undergoing biochemical re-evaluation at final height after withdrawal of rhGH therapy (persistent GHD-7, GH-sufficient-4) were recruited from Royal Hospital for Children between 2012-2013. By using further bone health assessment methods in addition to DXA (including p.QCT, mechanography, bone profiles and biomarkers), the bone density and body composition did not differ when we compared GHD to matched height but

normal GH at initial evaluation and retesting. However, naive GHD had lower muscle force as assessed by mechanography compared to the normal. In addition, bone resorption biomarker CTX was significantly higher in naive GHD vs. normal and that was significantly correlated to PTH levels in both first time assessment and retesting groups. Our results suggest that muscle force and serum PTH may be important determinants of bone health in subjects with CO-GHD.

Chapter 6 investigates lipids, adipokines (leptin- adiponectin- resistin) and glucose homeostasis and their relationship with bone and body composition in children and adolescents with CO-GHD at times of initial evaluation and retesting at final height (same population as chapter 5). Lipid profiles, adipokines and glucose homeostasis were not different between those with GHD and those who had normal GH levels across the groups of first time assessment and retesting. In the retesting group, those who were older at the time of diagnosis of CO-GHD with a shorter duration of rhGH therapy were more likely to have higher cholesterol($r=0.9$, $p<0.001$), leptin ($r=0.8$, $p<0.001$), and lower osteoclacin ($r=-0.7$, $p=0.01$) at final height. Leptin levels correlated positively with osteocalcin at diagnosis ($r=0.51$, $p=0.01$) but inversely at retesting ($r=-0.91$, $p<0.01$). The conclusion was that the timing and duration of childhood rhGH therapy might influence adiposity parameters and bone metabolism in subjects with CO-GHD.

In chapter 7 the study participants of chapter 5 were asked to complete either Short Form-36 (SF-36) or Adult Growth Hormone Deficiency Assessment (AGHDA) quality of life (QoL) questionnaires at the time of assessment of their GH axis. Our analysis showed that the overall QoL was not altered in children with naive GHD with a total score of SF-36 [93 (77, 96) naive GHD vs. 90 (84, 93) normal, $P=0.56$] (higher scores reflect better QoL). However, naive GHD had less energy and vitality scores compared with normal (75 (65, 100) vs. 95 (65,100) respectively, $p=0.04$), when the normal scored lower in the subscale of emotional well-being compared to those with naive GHD (78 (55, 84) vs. 90 (68, 96) respectively, $p<0.001$). In the retesting group, those with persistent GHD scored better in the AGHDA than GH sufficient (6 points (2, 8) vs. 9 points (7, 17) respectively, though not significant ($p= 0.10$) (higher scores reflect poorer QoL). Unexpectedly, subscale analysis showed that GH-sufficient subjects significantly lacked energy and complained of tiredness compared to those who were confirmed to have persistent GHD (5 points (3, 6) vs. 1 point (0, 1) respectively, $p= 0.03$). Further studies to validate QoL specific instruments in this population are needed with greater insight to elucidate factors that modify the relationship between GH status and QoL in children and adolescents.

Chapter 8 was a prospective intervention, randomised controlled study of 14 subjects among the first time assessment group (GHD-10, normal-4) and five subjects with CO-GHD among retesting group (persistent GHD-4, GH-sufficent-1). Subjects were randomised into either an exercise intervention group (EX) (25 jumps off 25 cm platform step/ three days /week for six months) or a control, in addition to rhGH being prescribed. The results of this study were limited by the small sample size and poor compliance. Therefore, there were insufficient data to recommend the use of weight bearing exercise in the absence of rhGH in children and adolescents with CO-GHD. Further

studies with adequate sample size that can more rigorously exam the optimal exercise interventions are needed.

Chapter 9 discusses the main findings of each chapter in this thesis and outlines potential limitations of the thesis methodology, and some important and interesting areas for future research in children and adolescents with CO-GHD.

Author’s Declaration

I hereby declare that all work presented in this thesis was performed entirely by myself, and was performed under the supervision of Dr M G Shaikh and Professor S F Ahmed.
No part of this thesis has been submitted in support of an application for another degree or qualification of this or any other University.

Dr Mahjouba Ahmid

I certify that the work reported in this thesis has been performed by Dr Mahjouba Ahmid and that during the period of study she has fulfilled the conditions of the ordinances and regulations governing the Degree of Doctor of Philosophy, University of Glasgow.

Dr MG Shaikh and Prof SF Ahmed

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"In the name of Allah Almighty, the most merciful, the most beneficent"
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Dedication

This thesis is dedicated to my husband Ihfaf Alshibane and my children Moussa, Maysam and Mutasim for they gave me values; enjoyment and love. Without their encouragement and understanding it would have been impossible for me to finish this work. I dedicate this work to them.

This thesis is also dedicated to my father Arhouma Emhammed and my brothers and sisters who have all supported me and given me the strength to complete this thesis.

This thesis is also dedicated to the living memory of my Mother Hafsa may Almighty ALLAH bless her.

Publications

Full Papers

1- **Ahmid M**, Fisher V, Graveling AJ, McGeoch S, McNeil E, Roach J, Bevan JS, Bath L, Donaldson M, Leese G, Mason A, Perry CG, Zammitt NN, Ahmed SF, Shaikh MG. An audit of the management of childhood-onset growth hormone deficiency during young adulthood in Scotland. Int J Pediatr Endocrinol. 2016;2016:6. doi: 10.1186/s13633-016-0024-8. Epub 2016 Mar 16.

2- **Ahmid M**, C G Perry , S F Ahmed, M G Shaikh. Growth Hormone Deficiency during Young Adulthood and the Benefits of Growth Hormone Replacement. Endocr Connect. 2016 May;5(3):R1-R11. doi: 10.1530/EC-16-0024. Epub 2016 Apr 29.

3- **Ahmid M**, S Shepherd1, M McMillan1,S F Ahmed1, M G Shaikh1 Bone Health, Body Composition and Metabolic Profilies, and Quality of life in Childhood Onset Growth Hormone Deficiency, in press.

Abstracts and Presentations

1- Audit of outcome of childhood onset growth hormone deficiency in young adults at the Royal Hospital for Sick Children, Yorkhill, Glasgow from 2005-2011 Poster presentation, British Society for Paediatric Endocrinology and Diabetes (BSPED), Leeds 2012 and Society for Endocrinology BES, Harrogate, 2012.

2-Management of childhood onset growth hormone deficiency in young adults. Oral presentation Scottish Paediatric Endocrine Group (SPEG), Dunkeld 2013

3- Management of Childhood-Onset Growth Hormone Deficiency in Young Adulthood. Poster presentation European Society for Paediatric Endocrinology (ESPED) Milan, 2013

4- Bone Mass and Body Composition in Adolescent with Childhood Onset-Growth Hormone Deficiency At Final Height Poster presentation British Society for Paediatric Endocrinology and Diabetes BSPED, Colchester 2014

5- Metabolic parameters and glucose homeostasis in children and adolescents with childhood-onset growth hormone deficiency at time of diagnosis and retesting. Poster presentation Glasgow paediatric research day 2015.

6- Bone Health and Body Composition in Childhood Onset Growth Hormone Deficiency at Time of Initial Evaluation and Retesting .European Society for Paediatric Endocrinology (ESPED 2016).

7- Metabolic Parameters and Glucose Homeostasis in in Childhood Onset Growth Hormone Deficiency at Time of Initial Evaluation and Retesting. European Society for Paediatric Endocrinology (ESPED 2016).

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Abbreviations

25 (OH) D	25-hydroxyvitamin D
AGHDA	Assessment in Growth Hormone Deficiency in Adults
A/G ratio	Android (trunk) /gynoid (legs) fat ratio
BA	Bone area
BAP	Bone-specific alkaline phosphatase
BL	Baseline
BMAD	Bone mineral apparent density
BMD	Bone mineral density
BMC	Bone mineral content
BMI	Body mass index
Ca	Calcium
CO-GHD	Childhood onset growth hormone deficiency
CSA	Cross sectional area
CTh	Cortical thickness
CTX	Cross-linked C-terminal telopeptide of type I collagen
DXA	Dual energy X-ray absorptiometry
EC	Endosteal circumference
EFI	Esslinger fitness index
EX	Exercise
FFA	Free fatty acid
F-max	Maximum - force
FM	Fat mass
FN	Femoral neck
FL	Follow up
GH	Growth hormone
GHD	Growth hormone deficiency;
GHBP	Growth hormone binding protein
GHR	Growth hormone receptors
GHRH	Growth hormone releasing hormone
HDL	High-density lipoprotein
HOMA-IR	Homeostasis model assessment insulin resistance index
IGF-1	Insulin-like growth factor 1;
IGHD	Isolated growth hormone deficiency
IGFBP	Insulin likes growth factor binding proteins
IL	Interleukin
ISCD	International Society for Clinical Densitometry
ITT	Insulin tolerance test

JAK2	Tyrosine kinase Janus kinase 2
LDL	Low-density lipoprotein
LM	Lean mass
LS	Lumbar spine
MAPK	Mitogen-activated protein kinases
Mg	Magnesium
MSC	Mesenchymal stem cells
MPHD	Multiple pituitary hormone deficiencies;
MRI	Magnetic resonance imaging
OC	Osteocalcin
OPG	Osteoprotegerin
OR	Odds ratio
PC	Periosteal circumference
PC%	Percentage change
p.QCT	Peripheral quantitative computed tomography
PO4	Phosphate
PICP	Carboxy-terminal propeptide of type I procollagen
PINP	Amino-terminal propeptide of type I procollagen
P-max	Maximum-power
PPV	Positive predictive value;
PTH	Parathyroid hormone
QoL	Quality of life
RANK	Receptor activator of nuclear factor κβ
RCT	Randomised controlled trial
RhG	Recombinant human growth hormone
SCOS2	Suppressor of cytokine signalling-2
SD	Standard deviation
SDS	Standard deviation score
SF-36	Short form-36
SSI	Stress-strain index
STAT	Signal transducers and activators of transcription
TB	Total Body
TG	Triglycerides
TNF-	Tumour necrosis factors-α
TrvBMD	Trabecular density
TvBMD	Total density
V-max	Maximum-velocity
WBE	Weight bearing exercise

CHAPTER 1

Introduction

1.1 Bone Biology

1.1.1 Bone structure

Bone is a dynamic connective tissue composed of bone cells (osteoblasts, osteocytes and osteoclasts), an extracellular matrix of collagen and non-collagenous proteins called osteoid, with inorganic mineral salts deposited within the matrix and water (1,2), (Table 1-1).
Histologically, bone can be classified as lamellar bone or woven bone. Lamellar bone is mature bone with collagen fibres arranged parallel to each other in lamellae around the medullary cavity where red bone marrow and/or yellow bone marrow (adipose tissue) is stored. The thickness of each lamellae is about 3-7 μm and is separated by an interlamellar layer around1μm thick (3).
Woven bone is immature bone, in which collagen fibres are arranged in irregular arrays (non-lamellar). Woven bone has poor mineral content and is formed during the early stage of fracture healing (3).
Bone can be also classified morphologically into two components according to tissue type: cortical bone and trabecular bone, (Figure 1-1).
Cortical bone (compact bone) is dense compact bone tissue which comprises 80% of the total bone mass of an adult skeleton. It is found in the shaft of long bones and as a thin layer covering other short and flat bones. Cortical bone is composed of cylindrical units called osteons (Haversian systems). The diameter of each osteon typically ranges from 20-110μm. Each osteon contains concentric lamellae layers of hard calcified matrix with osteocytes (bone cells) lodged in lacunae spaces between the lamellae. In the centre of each osteon, there is a central canal (Haversian canal) containg blood vessels and nerve fibres. Thiny canals (canaliculi) radiate outward from Haversian canal connecting the lacunae with each other and with the Haversian canal, providing nutrient exchange. Each osteon is in direct contact with the outer bone surface (periosteum), the bone marrow and other osteons via Volkmann's canals. Cortical bone porosity is around 3-5% in young individuals, and has less metabolic activity (3,4).
Trabecular bone (cancellous bone) comprises around 20% of the adult human skeleton and is found sandwiched between cortical bone layers such as in the interior of vertebrae and the head of the femur. Unlike cortical bone, trabecular bone does not contain osteons or Haversian systems. Instead, it is composed of a latticework of thin, mineralized irregularly shaped plates called trabeculae. Similar to osteon, trabecula has also osteocytes that lie in lacunae between calcified lamellae arranged parallel to each other, with bone marrow situated within this network providing the vascular and neural supply for the bone via diffusion from the inner bone surface (endosteum) lining the bone marrow spaces. Trabecular thickness is variable and ranges from 100-200μm(3,5). As in osteon, canaliculi present in trabeculae provide connections between osteocytes. However, since each trabecula is only a few cell layers thick, each osteocyte is able to exchange nutrients with nearby blood vessels. Trabecular bone rich in cancellous, is mainly responsible for the metabolic function of bone. Trabecular bone also has a high surface-to-volume ratio that is nearly

ten times the surface area of the cortical bone. This makes trabecular bone more prone for remodelling more than cortical bone with around 25% trabecular bone and 4% of cortical bone remodelling each year (6). The ratios of cortical bone to trabecular bone vary among different sites. For example, vertebrae are composed of cortical and trabecular bone in a ratio of 25:75. This ratio is 50:50 in the femoral head and 95:5 in the radial diaphysis (6).

Bone has also two surfaces that have different behavioural and functional properties: the periosteum and the endosteum. The periosteum is a thick fibrous membrane covering the external surface of cortical and trabecular bone (apart from its articular cartilage), and serves as an attachment for muscles and tendons. It consists of an outer layer of collagenous tissue containing a few fibroblast-like cells and an inner layer of fine elastic fibres with an osteogenic layer composed of osteoclast and osteoblast bone cells as well as mesenchymal cells. The inner layer of periosteum plays role in bone growth and metabolism as well as fracture repair. The periosteum is also enriched with nerves and blood vessels that innervate and nourish underlying bone by perforating (Sharpey's) fibres that extend from the periosteum into the bone matrix. The endosteum is a thin, vascular membrane that lines the medullary cavity and covers the trabeculae and may provide a barrier between the fluid within the canalicular and lacunar spaces and the extracellular fluid found in the marrow cavity and vessels. Like the periosteum, the endosteum has an osteogenic layer composed of osteoclast and osteoblast (3).

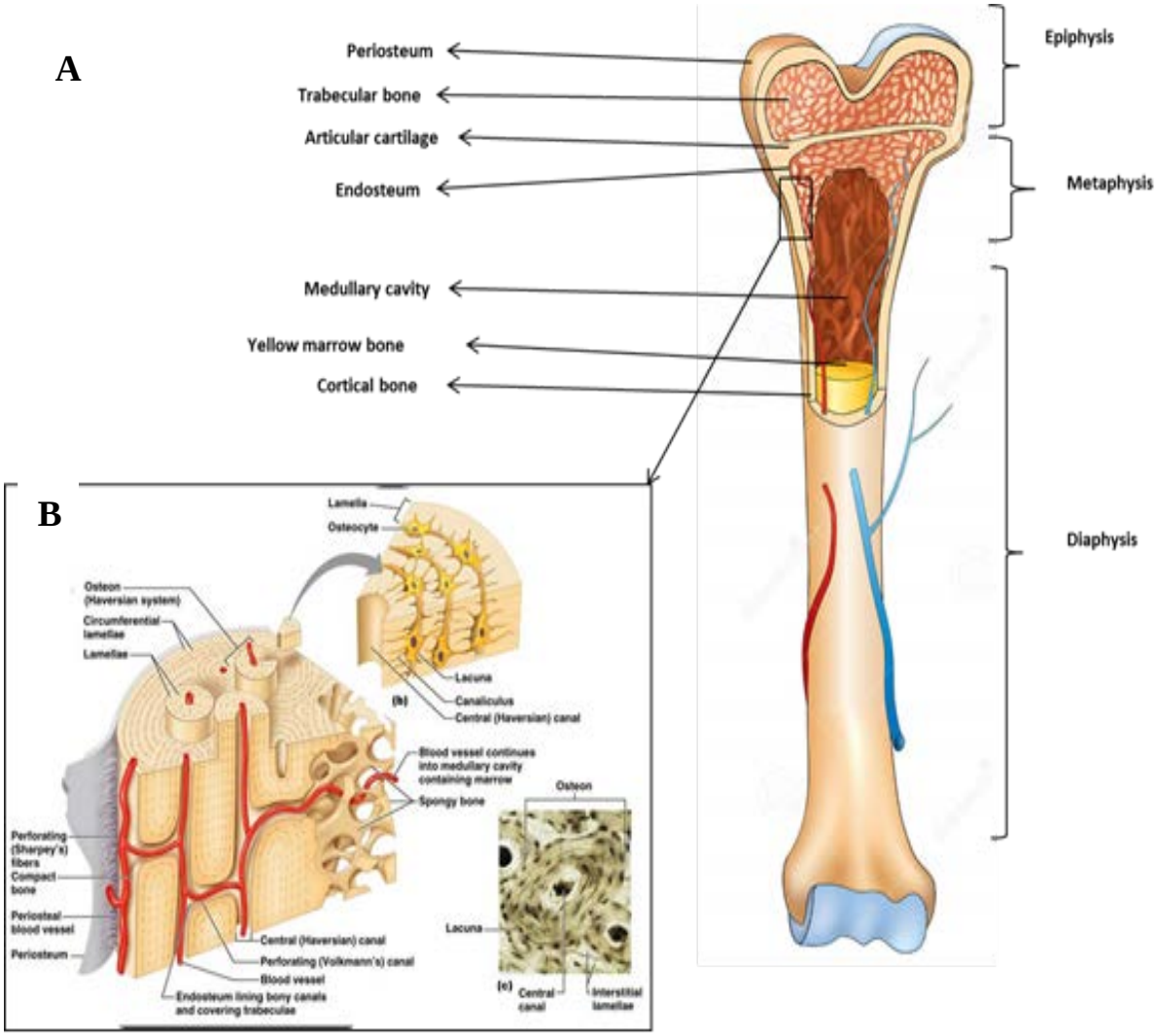


Figure 1-1 Bone macrostructure and microstructure

A- Bone macrostructure (adapted and modified from www.dreamstime.com)

B- Bone microstructure adapted from Copyright © 2004 Pearson Education, Inc., publishing as Benjamin Cummings

Table 1-1 Bone cells and extracellular bone matrix structure and function

	Location and Structure	Function
Bone cells		
Osteocyte	Star-shaped mature terminal differentiation stage of the osteoblasts, and the most common cell type in bone tissue (90% of bone cells).	Embedded in a mineralized matrix and thought to function as mechanosensors of mechanical loading. Osteocytes may undergo apoptosis, and may also be involved in the recruitment of osteoclasts and initiation of new bone remodelling.
Osteoclasts	Large (over 50 μm in diameter), multinucleated cells of hematopoietic origin.	The only cells that erode and resorb previously formed bone, and also dissolve mineral and release calcium and phosphorus into the extracellular fluid.
Osteoblasts	Large bone-forming cells (20-30 μm) in diameter derived from pluripotent mesenchymal stem cells.	Responsible for bone formation, synthesise and secrete unmineralised extracellular matrix (osteoid) and control the mineralisation with secreting vesicles containing alkaline phosphatase.
Extracellular matrix		
Organic		
Collagen	Consists primarily of type I collagen (~90%) in the form of fibrils oriented together in bundles throughout the tissue, and trace amounts of collagen types III and V.	Collagens and minerals together play an important function in the biomechanical properties and functional integrity of bone. Collagen proteins are organised in a preferential way in order to increase bone toughness and reduce the risk of fracture.
Non-collagen	It form 10% of the organic matrix and is made up of non-collagenous proteins, such as bone sialoprotein, osteonectin, osteopontin, osteocalcin, matrix-GLA-protein, and others.	May play a role in binding the collagen and minerals together, and is involved in regulating bone mineralization and remodelling.
Inorganic		
Bone minerals	Calcium and phosphorus in the form of an insoluble salt called hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$], in addition to carbonate, citrate, fluoride, chloride, sodium, magnesium, potassium and strontium.	Bone stores 99% of the body's minerals, which provide hardness to the bone, and play a role in keeping blood levels within a narrow range for normal physiological functioning.

1.1.2 Bone modelling

Bone modelling refers to a process in which bones are shaped or reshaped by the independent activities of osteoclasts and osteoblasts. Bone modelling involves three stages of the production and maturation of osteoid matrix, followed by mineralization of the matrix. As a result, bone tissue increases by expansion of the marrow cavity following matrix deposition. By the end of modelling, 50-70% of osteoblasts remain on the surface as inactive flat lining cells, and around 15% of mature osteoblasts become embedded in new bone matrix to differentiate into osteocytes (7). Bone modelling differs from bone remodelling, because this process at a single site involves bone formation that is not coupled with prior bone resorption. The modelling process is most pronounced during bone growth and development, which is determined in part by genetics (8), and is less frequent than remodelling in adults (9,10).

1.1.3 Bone remodelling

Bone remodelling is defined as the process of bone being renewed to maintain bone strength and mineral homeostasis throughout life by removing and replacing skeletal structures during growth or adaptive responses to a change in mechanical loading patterns. Bone remodelling involves sequential activities conducted by complex coordination between osteoblasts, osteoclasts, and osteocytes which are known as basic multicellular units (BMUs) within bone remodeling compartment (BRC). However, recently, more cell types have been identified to contribute to remodelling (such as T-cells, macrophages, and precursor populations of osteoblasts and osteoclasts) (11). The bone remodelling cycle can be divided into the following phases:

The activation phase involves retraction of the bone lining cells, recruitment and activation of osteoclast precursors from the circulation to produce osteoclasts at a quiescent bone surface. During this stage, osteoblast-lineage cells (osteocytes, lining cells and preosteoblasts cells) produce receptor activator of the NF-κB ligand (RANKL), a member of the tumour necrosis factor (TNF) family, and macrophage colony stimulating factor (MCSF) drive the differentiation and survival of the osteoclast precursors into osteoclasts. RANKL can be activated by binding to RANK receptors on the cell membrane of osteoclast precursors and is inhibited by osteoprotegerin (OPG), which is a glycoprotein secreted mainly by osteoblast lineage cells. RANKL/RANK/OPG system is the key regulator of the bone resorption cycle that bone resorption and bone formation are coupled among others (6).

The resorption phase is the stage when osteoclasts begin acidification and dissolution of the mineral matrix and decomposition of the osteoid matrix. Osteoclast resorption is by means of releasing enzymes cathepsin K, matrix metalloproteinase-9 (MMP-9) and tartrate resistant acid phosphatase (TRAP) to break down the organic components (12). This process takes around two to four weeks during each remodelling cycle.

The reversal phase occurs at the end of resorption phase. Mononucleated cells (reversal cells including monocytes, osteocytes) appear at the same resorption site, cover the bone surface and prepare the surface for new osteoblasts to begin bone formation, and provide signals for osteoblast precursor's differentiation and migration (1). During this phase, osteoblasts interact and communicate with osteoclasts in a process known as 'coupling' that allows transtion from bone resorption to bone formation phase. The coupling may be controlled by several factors including matrix-derived factors (such as tansforming growth factor β (TGF- β) and insulin-like growth factor-I; osteoclast secreted factors (such as sphingosine-1-phosphate, Wnt 10b, BMP-6); and osteoclast membrane bound factors (such as EphrinB2 and Semaphorin D) (13,14). This stage may last up to four or five weeks.

In the formation phase, osteoblasts fill the resorptive cavity by depositing newly synthesized bone matrix. The osteoblasts first secrete the unmineralised osteoid, which is composed of 90% type I collagen; the remaining 10% is made up of some minor types of collagen, proteoglycans and specific bone proteins such as osteopontin, bone sialoprotein and osteocalcin. Subsequently, osteoblasts trigger mineralisation of new matrix by releasing matrix vesicles and initial mineral deposition. This is a longer stage of the remodelling cycle that can continue for four months until the new bone structural unit is completed. On completing this phase, osteoblasts undergo apoptosis so the surface is covered with flattened lining cells and a prolonged resting period begins until a new remodelling cycle is initiated (8), (Figure 1-2).

Approximately 20% of bone tissue is replaced annually, varying by site and type through the remodelling process (15). The majority of bone remodelling occurs on the surface of bone in the trabecular bone (60%) and takes approximately seven months in trabecular bone compared with four months in cortical bone (8). After peak bone mass is attained, a very small percentage of bone is lost with each remodelling cycle, such that over one year approximately 0.5 to 1% of the total body bone mass is lost (6).

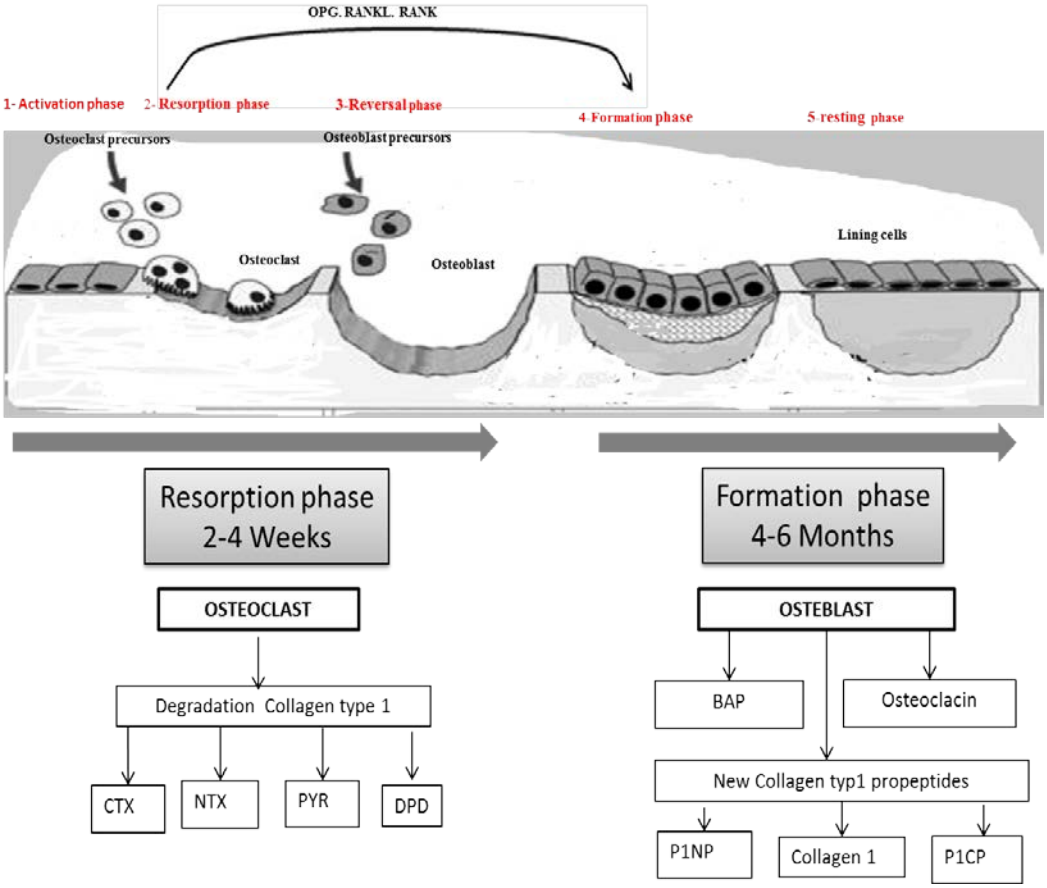


Figure 1-2 Bone remodelling cycle.
Bone formation markers include: bone alkaline phosphatase (BAP), terminal propeptides of procollagen type I (PINP, PICP), and osteocalcin. Bone resorption markers include: Collagen type 1 cross-linked C-telopeptide (CTX) and N-telopeptides (NTX) pyridinoline (PYD) and deoxypyridinoline (DPD). See the text for more details.

1.1.4 Bone metabolism biomarkers

Bone metabolism biomarkers are specific bone-derived molecules that reflect enzymatic activity of the bone cells and bone turnover. Bone metabolism biomarkers can be classified into two categories: bone formation and bone resorption markers. Both physiological (such as age, puberty or growth velocity) and pathological conditions can cause significant changes in the concentrations of bone markers (16,17). The balance between bone resorption and formation (bone turnover) is regulated by several factors, mainly genetic (70%), in addition to mechanical, vascular, nutritional, hormonal, and local factors as summarized in Table 1-2. The imbalance between bone resorption or bone formation can lead to several pathological bone diseases such as osteoporosis and Paget's disease.

High bone turnover and increased osteoclast activity predisposes to increase bone loss, decrease trabecular and cortical thickness and increases in porosity, whereas, low bone turnover and reduced bone formation is associated with reduced trabecular penetration and erosion and relative preservation of bone micro architecture (18). In clinical practice, bone metabolism biomarkers are used for therapeutic decision making in osteoporosis and therapeutic monitoring, but their use alone to predict fracture risk has yet to be established (19). In paediatric practice, bone biomarker measurement may be interpreted relative to age and sex reference curves (20). Some of the widely used types of bone biomarkers and those used in this thesis are detailed in the next section.

Table 1-2 Factors stimulate bone formation and bone resorption.
Summarized from reference (8)

	Stimulate formation	Stimulate resorption
Local factors		
Cytokines / Adipocytokines	IL-4, IL-3, IL-18 OPG, TNF-β, Adiponectin	TNF-α, IL-1, IL-6, IL-8, IL-11, PG, Leptin
Growth factors	BMP-2, BMP-4, BMP-6, BMP-7, IGF-1, IGF-2, TGF-β, FGF, PDGF	EGF, M-CSF, GM-CSF, PDGF, FGF
Systematic factors		
PTH*	↓	↑
VIT-D	↑	↓
GH	↑	↑
T3,4	↑	↑
Oestrogens	↑	↓
Glucocorticoid	↓	↑
Calcitonin	-	↓
Genetic factors	+	
Mechanical factors	+	

Bone morphogenic protein (BMP), Insulin-like growth factor1 -2 (IGF-1, IGF-2), transforming growth factor (TGF), fibroblast growth factors (FGF) and platelet-derived growth factor (PDGF), epidermal growth factor (EGF), macrophage colony-stimulating factor (M-CSF), Granulocyte macrophage colony-stimulating factor (GM-CSF), tumour necrosis factors-α, β (TNF-α, β), Interleukin (IL-1,3,4,6, 8,11,18) and prostaglandins (PG), osteoprogestrin(OPG), parathyroid hormone(PTH), growth hormone(GH), thyroid hormones (T3,T4).

* PTH action on bone formation markers and resorption markers is dependent on PTH secretion patterns

1.1.4.1 Bone formation markers

Bone formation markers reflect osteoblast activity in the process of bone formation and can be measured in serum or plasma. The most commonly used markers of bone formation are bone alkaline phosphatase and osteocalcin which are released at different stages of osteoblast proliferation and differentiation.

1.1.4.1.1 Bone alkaline phosphatase (BAP)

Alkaline phosphatases (ALP) are a group of isoenzymes encoded by four genes that code for non-specific, intestinal, placental and placenta-like isoenzymes. The majority of total serum alkaline phosphatases in serum is produced by the same gene from bone and liver which differ only by post-translational glycosylation (21). In adults with normal liver function, approximately 50% of the total ALP activity in serum is derived from the liver, while 50% arises from bone. In children and adolescents the bone-specific isoenzyme predominates (up to 90%) because of skeletal growth (22). Current available immunoassays allow simple and rapid quantitation of either enzyme activity or enzyme mass. However, these assays still have cross-reactivity with the liver isoenzyme of 15–20% (23). The bone isoform of ALP (BAP) is produced by osteoblasts in the bone and released in high amounts into the circulation during the early stage of bone formation cycle. The precise function of the enzyme is yet unknown, but it obviously plays an important role in osteoid formation and mineralisation (23). BAP has two identified major isoforms (B1 and B2) specified for cortical and trabecular bone activity respectively, and two minor isoforms (B/I and B1x) (24). However, whether certain isoforms are more prevalent in certain clinical conditions has not yet been determined. BAL levels during childhood correlate positively with age and height velocity as well as puberty in both genders (25). Serum levels of BAP show no significant circadian variations (26) and it has a long half-life (1-2 days) (27). Therefore, BAP level assays have been considered to be the most widely available, inexpensive marker of bone formation markers.

1.1.4.1.2 Osteocalcin

Serum osteocalcin (OC) is a 5.8 kDa, hydroxyapatite-binding protein that is exclusively synthesized by the osteoblasts and is considered the most accurate marker of bone formation. OC is also known as bone Gla protein because it contains three vitamin K-dependent γ-carboxyglutamic acids (Gla) residues, which serve as calcium binding sites and may be involved in bone mineralization (23). OC accounts for about 1% of the total bone proteins and is the most abundant non-collagenous bone matrix protein, with larger amounts found in cortical bone (25). OC can be considered a specific marker of osteoblast activity in the process of osteoid mineralisation with around 10-40% of OC released into circulation during the bone matrix mineralization phase (28). OC can be found in two forms: carboxylated OC accumulates in bone and tightly binds to Ca ions in hydroxypatite, and decarboxylated OC is the most circulator and active form of OC but has less affinity for Ca. However, in addition to the intact molecule, several forms of immune reactive

fragments of various size of OC have been identified in circulation (29). It has also been suggested that OC (N-terminal isoform) may be released during bone resorption phase and be involved in feedback regulation of bone remodelling (30). Recent animal studies suggest that decarboxylated OC is inversely related to increased insulin resistance and visceral adiposity and is involved in regulation of energy metabolism through stimulation of insulin secretion and production by pancreatic β-cells (31,32). There is limited evidence to support the association between OC and human glucose metabolism from clinical studies (33).
OC has a short half-life of about five minutes, with significant circadian variations with a nocturnal peak and a drop of about 30% towards morning (34). For OC instability, there are pre-analytical cautions that the sample be collected on ice, separated within one hour and frozen under −20 °C for short-term storage and at −70 °C for longer term storage (23).

1.1.4.2 Bone resorption markers

Bone resorption markers are products of bone collagen degradation mediated by osteoclasts. Five types of bone resorption markers have been established: Collagen cross-linked molecules (pyridinoline and deoxypyridinoline), pyridinium cross-links, cross-linked telopeptides of collagen type 1, hydroxyproline, and hydroxylysine. During bone resorption, 60% of bone matrix degradation products are released in urine in the form of peptide-attached cross-links and 40% as free fraction pyridinium crosslinks (21).
More commonly used assays use antibodies against amino acid sequences within the collagen type 1 C- and N-terminal telopeptides (CTX and NTX, respectively) in serum and urine.

1.1.4.2.1 Collagen type 1 cross-linked C-telopeptide(CTX)

CTX is cleaved from type 1 collagen by cathepsin K activity during bone resorption. CTX is reported to be more specific to bone resorption than other markers (35). There are two specific types of CTX isomers: non-isomerized (αCTX) and beta-isomerized (βCTX) forms which presumably measure degradation of relatively young and old bone respectively (36).
There is also evidence that CTX can be used as an early marker of bone mass in response to treatment, with 49.5% sensitivity for detecting improvement in bone density (37). However, the major disadvantage of CTX is its large circadian variation (38) and a high dependence on fasting status, necessitating a morning fasting sample for accurate interpretation (39). According to several published references, CTX levels are highest in neonates and then markedly decrease in children after one year of age. A second peak is observed in girls aged 11–13 years and in boys aged 14–17 years, which coincides with the pubertal growth spurt (25).

1.1.5 Bone growth and development

Bones grow and change across the lifespan, predominantly during childhood with longitudinal linear growth followed by intramembranous bone growth and expansion. There are several genetic and epigenetic factors that influence and regulate bone growth and development. Genes regulate cell differentiation and ultimately the morphogenesis of bone. Genes produce transcription products that are translated via signalling pathways into regulatory, enzymatic, or structural proteins (40). Epigenetic factors that occur following genetic determination include regulation, systemic and local bone growth factors, in addition to mechanical control factors (41,42) as listed in (Table 1-3).

Table 1-3 Systemic and local bone growth factors regulate bone growth and development. Summarised from reference (42)

	Systematic factors	Local factors
Linear	Growth hormone (GH), insulin-like growth factor 1 (IGF-1), thyroid hormones (T3) and (T4), sex steroid, and glucocorticoids (suppressed).	Parathyroid hormone–related peptide (PTHrP), and fibroblast growth factors (FGFs), indian hedgehog (Ihh), bone morphogenetic proteins (BMP), vascular endothelial growth factor (VEGF), cytokines, growth factors, and prostaglandins. In addition to tension and mechanical factors (body weight-bone length)
Width	Sex steroid (Androgen stimulated: oestrogen suppressed), GH, parathyroid hormone	Genetic, mechanical force

1.1.5.1 Longitudinal growth (linear)

Bone linear growth occurs mostly during childhood by enhancing chondrogenesis and ossification at the end plates of long bones. This involves first proliferation and then differentiation of mesenchymal cells into pre-chondroblasts and then into chondroblasts instead of osteoblasts within the proliferative zone, in between the growth plate's reserve zone and the zone of provisional calcification (43). Hypertrophic chondrocytes alter the structure of the surrounding cartilage matrix and is involved in the degradation of most of the cartilage matrix. This alteration allows the vascularisation and secretion of woven bone matrix and mineralisation adjacent to the remaining columns of chondrocytes while degraded cartilage matrix is replaced by collagen X (41). The rate of longitudinal bone growth is controlled by genetic, hormonal and biomechanical factors, in addition to numerous systemic and local growth mediators which all contribute to establishing the final height of an individual (42). The importance of GH and IGF- 1 in stimulating longitudinal growth has long been established. GH is considered as the main stimulator of chondrocyte proliferation in the growth plate, while IGF-1 is understood to act mainly on post-proliferation in the hypertrophic zone and in chondrocyte differentiation (44).

1.1.5.2 Intramembranous growth (width)

Bone growth in width is one of the most important determinants of bone strength throughout life. Bones grow in width through periosteal expansion as a result of osteoblasts adding new bone matrix on the outer or periosteal surface which is later mineralized. At the same time, osteoclasts located on the inner (endocortical) surface of the cortex resorb bone, thus increasing the size of the marrow cavity (7,41). Periosteal expansion of diaphyseal cortical bone is suggested to be exclusively exerted by circulatory IGF-1 (45).

1.1.5.3 Bone growth during puberty

Puberty is a dynamic period marked with rapid changes in bone size and structure of both males and females. Bone changes during puberty occur dominantly in the spinal regions and are characterised by gender differentiation under the influence of sex steroids. In boys, periosteal apposition expands and increases the bone width while endosteal resorption enlarges the medullary cavity. Cortical thickness increases because periosteal apposition is greater than endocortical resorption. In girls, periosteal apposition decelerates earlier, and there is no change in medullary size in girls at some sites, but there is medullary contraction at other sites (46,47). As a result of decelerated periosteal apposition and contraction of the medullary cavity in girls, bones become smaller in width and in medullary size than in boys, but with a similar cortical thickness and bone size (48).

1.1.6 Bone strength

Bone strength is generally defined as the maximal bone resistance to external load without yielding or fracture (49). Although bone strength is largely determined by genetic factors, it could generally be influenced by a combination of several factors that determine bone quantity and quality, including geometry (size and shape), bone density (BMC-BMD), microarchitecture (trabecular/cancellous architecture and cortical thickness/porosity), and bone structure (mineral and collagen composition) (50), (Figure 1-3).

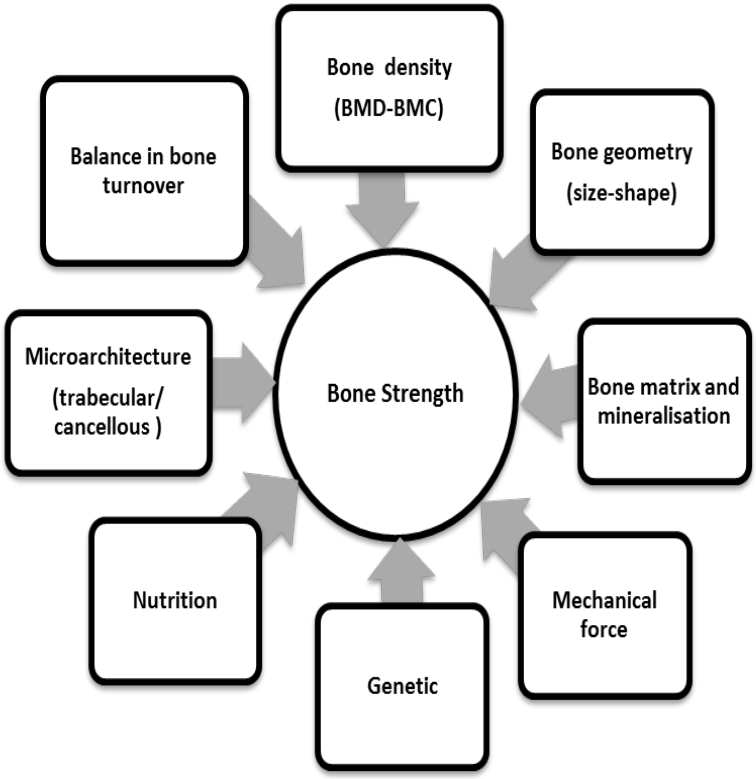


Figure 1-3 Factors determine bone strength

1.1.6.1 Bone density and geometry

Bone mass and density account for 66 to 74% of bone strength (49). Bone mass is described as a result of two processes: firstly, acquisition of bone mass during childhood and, secondly, maintenance of the existing bone mass throughout life (51). Genetic factors account for 50-80% influence on bone mass, in addition to nutrition, physical activity, height, weight, and hormonal status (GH, sex steroid) (52).

Compartment density (bone BMD) has been widely used as a noninvasive surrogate of bone mass as well as a predictor of fracture risk (53). BMD can be measured by non-invasive imaging modality such as dual-energy X-ray absorptiometry (DXA) and peripheral quantitative computer tomography (pQCT). DXA is the most commonly used method in assessment of BMD but only takes two-dimensional areas into account (areal BMD), disregarding bone depth (54), while pQCT is a research tool used to assess volumetric BMD of cortical and trabecular components (55). In addition to bone density, bone size and its geometry is an important factor in determining distribution of bone mass and the ability of bone to resist bending and torsional loading (49,56).

1.1.6.2 Bone microarchitecture

Bone microarchitecture makes an important contribution to bone strength (57,58). Bone microarchitecture assessment is based on a measure of trabecular bone number, thickness and separation, as well as their spatial organization in the marrow space. The combination of architectural features with bone volume explain > 90% of bone strength (59). There are few methods currently clinically validated to assess and monitor bone microarchitecture, including 3D non-invasive scanning methods such as high-resolution peripheral quantitative computed tomography (HR-pQCT), and nonionizing high-resolution MRI (58).

1.1.6.3 Bone matrix and microfractures

The optimal balance in bone matrix elements (mineral homeostasis and collagen) and microstructure are known to play important roles in skeletal development and strength (56). Regulation of bone mineral (calcium, phosphate and magnesium) homeostasis occurs at three target tissues, kidney, intestine, and bone, principally via the complex integration of two chemicals, parathyroid hormone (PTH) and vitamin D. Calcium metabolism plays an important role in bone turnover and deficiency of either calcium or vitamin D interferes with mineral deposition (60). Magnesium, in addition to its role in living cells including bone cells, is an important contributor in bone health through alteration of the structure of hydroxyapatite crystals and its release follows the resorption of bone (61).

PTH is known to maintain calcium within a narrow range through its actions on both kidney and bone (62). In addition, PTH has dual effects on bone metabolism through stimulation both bone formation and bone resorption depending on the pattern of its secretion (62,63). It has been suggested that intermittent PTH secretion stimulates bone formation by reactivation of lining cells to become active osteoblasts and promoting osteoblastogenesis and survival of mature osteoblasts to prolong their matrix synthesizing function. In contrast, continues PTH indirectly promotes osteoclast formation and bone resorption by increasing RANKL and inhibiting OPG through its actions on osteoblast and osteocytes (64).

On the other hand, the accumulation of microfractures and suppression of its repair may contribute to bone weakness and fragility (65). The degree of bone mineralization and microfractures is dependent on bone turnover. Low bone turnover leads to an accumulation of microfractures, but there is more time for mineralization to proceed, while excessive bone turnover with greater bone resorption than formation leads to microarchitectural deterioration (65).

1.1.6.4 Muscle force and function

Based on the Mechanostat theory (66,67), it is now widely accepted that skeletal muscle contraction imposes a major mechanical stimulus for bone development, suggesting a coupling of the muscle-bone function unit indices of bone strength (68). Based on this concept, bone adapts its geometry and strength to withstand challenges from maximum muscle force during growth (69). The adaptation of bone to muscular forces (musculoskeletal interaction) may serve as a useful tool in differentiating between different pathogenetic pathways of endocrine and metabolic bone diseases (68). Muscle function can be defined as the coordinated contraction of individual muscle fibres within each skeletal muscle generating muscle force or power (70). Muscle force cannot be measured directly in clinical studies, but there are several parameters implied to reflect maximal muscle force either through measuring muscle mass (kinematics) or muscle force (kinetics) which is a more reasonable reflection of maximal force(71). Several studies explored the utility of isokinetic dynamometry using a hand force grip device in assessing muscle torque in the paediatric population (72). However, hand force grip has been known to have low reliability limited its clinical applications. Therefore, much higher peak forces have been measured for eccentric contraction at the shaft using a ground reaction force platform (GRFP; approx. 10•5 times body weight) than isokinetic dynamometry (approx. 4•8 times body weight) (73). Fricke et al. (74) introduced a jumping mechanographic device to measure muscle function and power which derive from individual ground reaction forces in children and adolescents. Since then, several published studies have validated Mechanography in measurement of muscular function in children, adolescents and adults (75-78).

1.1.6.5 Bone strength and osteoporosis

Osteoporosis is a common disease defined by the World Health Organization (WHO) as a "systemic skeletal disease characterized by low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fractures"(79). In adults, definition of osteoporosis is based only on the lumbar spine /femoral neck BMD of DXA scan which has to be -2.5 standard deviations or lower than the average bone mass of healthy young adults (T-score) to enable a diagnosis (80,81). However, definition and diagnosis of osteoporosis in children and adolescents is less established than in adults. In clinical practice, diagnosis of osteoporosis in children and adolescents requires the presence of a clinically significant fracture history and low BMC or BMD. A clinically significant fracture history is defined as one or more of the following: long bone fracture of the lower extremities, vertebral compression fracture or two or more long bone fractures of the upper extremities. Low BMC or BMD is defined as a BMC or areal BMD Z-score that is less than or equal to −2.0, adjusted for age, gender and body size, as appropriate. A Z-score between −1.0 and −2.0 is defined as the low range of normality (82). Recently, the International Society for Clinical Densitometry (ISCD) issued

statements specifying to use low bone mass and density rather than osteoporosis in the absence of a history of clinically significant fractures (83).

As in adults, osteoporosis in the paediatric population can be classified according to aetiology into primary osteoporosis and secondary osteoporosis. Primary osteoporosis is rare but it can be idiopathic or related to hereditary disorders of connective tissue such as osteogenesis imperfecta. Secondary osteoporosis is related to endocrine disorders (hypogonadism, hyperthyroidism, vitamin D deficiency, and primary hyperparathyroidism), gastrointestinal disorders, genetic diseases or medication. These causes have their own pathogenesis, epidemiologic features, and effects on bone quality (84). Osteoporosis generally develops by three main mechanisms: inadequate bone mass, excessive bone resorption, and inadequate formation of new bone during remodelling (85).

1.1.6.6 Growth hormone deficiency (GHD) and osteoporosis

GHD is a heterogeneous disorder, and skeletal manifestations in patients developing GHD in their childhood are different than in patients developing the disease after they have reached their final height (86). Histomorphometric data of bone biopsies taken from adults with GHD revealed a decrease in osteoid and mineralizing surfaces and low bone formation rate (87). Animal model studies of null GH/IGF-1 knockout mice suggested GHD impaired periosteal bone formation, but had limited impacts on trabecular bone structure and density (44). Likewise, clinical studies of children with CO-GHD showed a reduction in bone turnover (88) and lower cortical bone (89) at first diagnosis, and in the long term if left untreated (90). Furthermore, studies of adults with GHD have proposed abnormalities in the circadian rhythm of PTH in GHD, which may affect bone remodelling (91). Based on these different mechanisms, there is still a lack of clear evidence that GHD could increase fracture risk or be a direct causes of osteoporosis (92) as will be described in this thesis.

1.2 Growth Hormone (GH)

1.2.1 GH Physiology

GH is a polypeptide hormone, 80% of which consists of a 22 kDa (kilodalton) single-chain α–helical non-glycosylated polypeptide with 191 amino acids and two intra-molecular disulfide bonds. However, due to alternative splitting, a short form of 20 kDa (10-20%) is also produced. The GH is encoded by two genes on the long arm of chromosome 17 (called GH-N or 1 in the pituitary and GH-V or 2 in the placenta).

1.2.2 GH secretion and neuroendocrine regulation

1.2.2.1 GH secretion

GH is secreted by the anterior pituitary somatotrope cells and has a half-life of 20-30 minutes (93). GH is detectable in the human foetal pituitary as early as 12 weeks gestation at a level of 20 ng/ml, increases to a maximum of approximately 80 ng/ml at 22 weeks gestation and then declines at term to approximately 10 ng/ml (94). Thereafter, GH is secreted in a pulsatile pattern with the highest rate at the onset of puberty and reaching 2-3 times the prepubertal level by mid and late puberty. This occurs earlier in girls than in boys because of the effects of sex steroid hormones (93). Healthy non-obese young adults' spontaneous physiological GH secretion is 0.25+0.03 mg/m^2 of body surface per 24 h (0.4–0.5 mg/24 h) (95). After the age of 20 years and thereafter, the secretion of GH starts to decline with the age and body mass index (BMI) up to 14% each decade and 6% for each unit increases in BMI (96).

1.2.2.2 GH neuroendocrine regulation

GH secretion is regulated by two peptides from the hypothalamus, GH releasing hormone (GHRH) which stimulates GH release and somatostatin (SS) which inhibits GH release. These peptides are secreted into sinusoidal capillaries of the median eminence and reach the anterior pituitary gland via its portal veins. An intact interaction between these two peptides is needed for the GH pulsatility pattern (97). Recently, researchers have suggested ghrelin, a peptide released by gastric cells in the stomach, participates in the pulsative regulation and enhancement of GH secretion (98). In addition to hormonal regulations, GH secretion is also influenced by several neural, metabolic and pathophysiological factors (99), as summarised in Table 1-4.

Table 1-4 Factors affecting GH secretion

	Stimulation	Inhibition
Physiology	-Sleep -Exercise -Stress	- Psychological stress
Hormonal	- GHRH - Ghrelin -Hyperthyroidism - α2-adrenergic agonists and cholinergic agents, and dopamine agonist - Sex steroid (Oestrogen)	- Somatostain - IGF-1 -Glucocorticoids -Hypothyroidism
Metabolic	-Hypoglycaemia -Amino acid (arginine)	-Hyperglycaemia -Fatty acid
Pathological	-Renal failure -Anorexia nervous -Acromegaly	-Obesity

1.2.3 Growth hormone binding protein and receptor signalling

1.2.3.1 GH-binding protein (GHBP)

Secreted GH circulates both unbound and bound to GH binding protein (GHBP), which is a portion of the extracellular domain of the GH receptor (GH-R). GHBP is produced mainly by the liver and other tissues (100). Approximately 50% of circulating GH is bound to GHBP, whereas free GH depends much on prevalent total GH and GHBP concentrations (101). GHBP acts as a circulating buffer or reservoir for GH, prolonging the half-life of plasma GH and competing with GH-R, of which the extra membranous portion is identical to GHBP. Levels of GHBP reflect the level and activity of GHR (102).

1.2.3.2 GH receptor (GH-R) signalling

GH-R, which is a 620-amino-acid cytokine protein that has been identified in many tissues including muscle, fat, liver, heart, kidney, brain and pancreas (103). Signal transduction starts when GH binds to the extracellular domain of its receptor; each single GH molecule binds to two GHRs, in two asymmetric sites. Binding one GH molecule to two GHR molecules leads to the activation of receptor-associated Janus kinase (JAK) 2 and subsequent cross-phosphorylation of tyrosine residues in the kinase domain of each JAK2 molecule, followed by tyrosine phosphorylation of the GHR. This phosphorylation leads to further phosphorylation of the Signal Transducers and Activator of Transcription (STAT) protein, activating the STAT pathway (104).

Activation of the STAT pathway, in particular STAT5b, results in the activation of several genes that bring about stimulation of osteoclast differentiation, epiphyseal growth, lipolysis and amino acid uptake into muscle (103,105). GH-R signalling has also been associated with the activation of phosphatidylinositol 3-kinase (PI3K), and the mitogen-activated protein kinase (MAPK) pathways. These two pathways have been linked with increases in protein synthesis and inhibition of protein degradation, and mediate the various metabolic and mitogenic responses elicited by insulin and IGF-1 (103). The JAK-STAT pathway of GH signalling is negatively regulated by the cytokine-inducible Suppressor of Cytokine Signalling (SOCS) family (mainly SOCS-1, -2, -3 and CIS (cytokine-inducible SH2-containing protein)) through binding to JAK2 and to certain cytokine receptors and signalling molecules, thereby suppressing cytokine signalling and terminating the GH signal (106), (Figure 1-4).

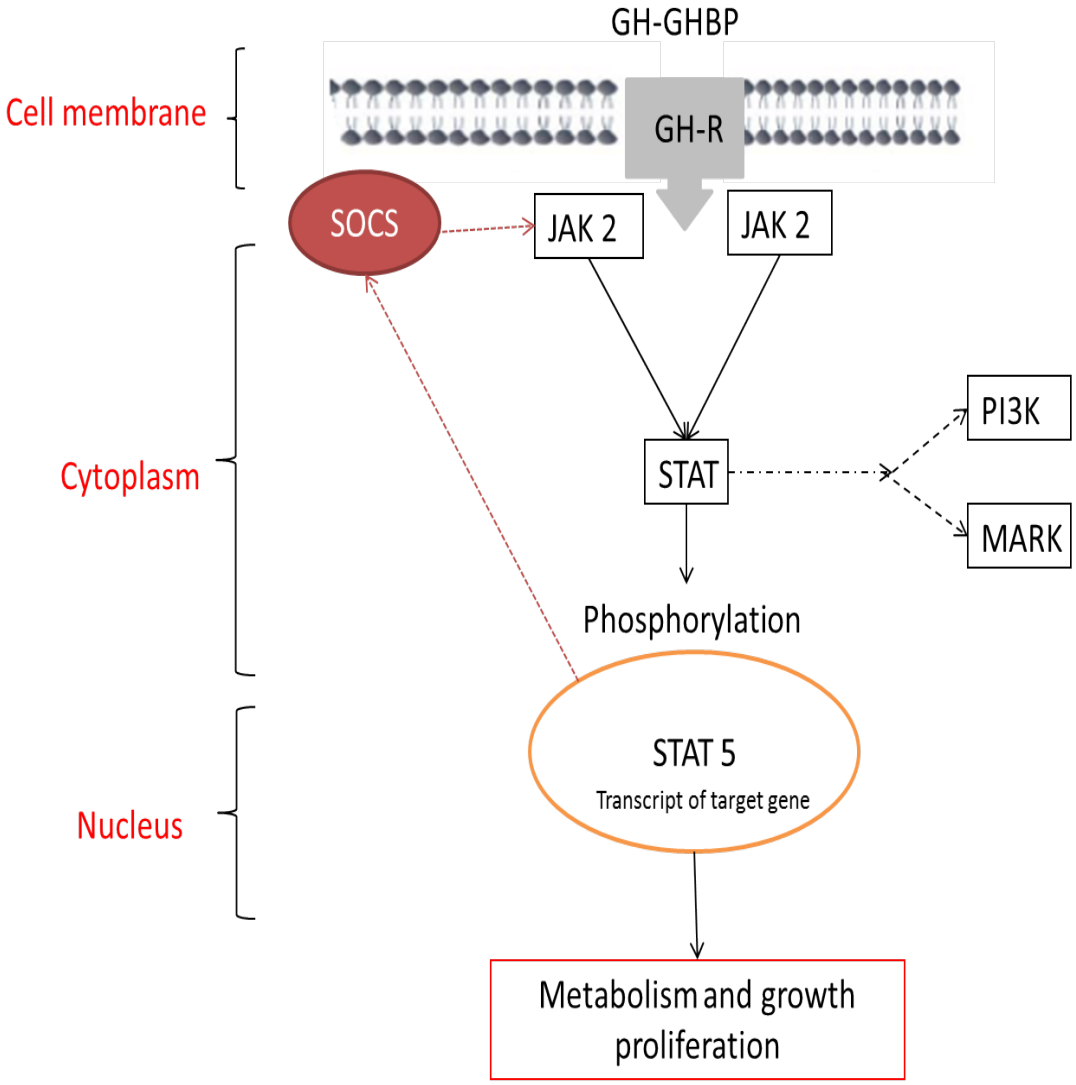


Figure 1-4 Intracellular pathways involved in growth hormone signalling.

Binding of GH-GHBP to GH-R activates Janus kinase 2 (JAK2), signal transducer and activator of transcriptions (STATs particular 5).JAK2 also activates other signalling pathways, such as mitogen-activated protein kinases (MAPKs) and phosphoinositol 3kinase (PI3K). Suppressors of Cytokine Signalling (SOCS) are the negative regulators of GH-GHR action. Solid and hutched black arrows indicate GH signalling pathways, Red hatched arrows indicate pathway negatively regulated GH signalling

1.2.4 Insulin-like growth factor system

The insulin-like growth factor system consists of insulin-like growth factors (IGF-1, IGF-2) with six IGF-binding proteins (IGFBPs) and IGF-1 receptors (IGF-1R). Insulin-like growth factor 1 (IGF-1), also called somatomedin C, is a peptide hormone which has 50% similarity in amino acids to proinsulin and insulin (107). IGF-1 is produced under the direction of GH predominantly in the liver (circulatory-IGF-1), and is also secreted locally by other tissues (bone and muscle) where paracrine and autocrine signalling take place(local-IGF-1) (108). Approximately 99% of circulatory IGF-1 is bound to one of the six IGFBPs, with around 90% of total serum IGF-1 binding to IGFBP-3 on the ternary complex with an acid labile sub-unit (ALS) (109). Both IGFBPs and ALS are produced mainly by the liver and act as a reservoir for IGF-1, increasing their plasma half-life from 10 minutes to 3-4 hours. They transport IGF-1into target cells, and modulate the interaction of IGF-1 with its receptor to exert IGF-1-independent effects (109). The majority of IGF-1-dependent actions are mediated mainly by the union of IGF-1 to IGF-1R, which has tyrosine kinase activity. Upon binding, the IGF-1R undergoes auto-phosphorylation and creates phosphorylated tyrosine residue signals through the PI3K pathway (110). IGF-1 also binds with low affinity to the insulin receptor and shares its hypoglycemic effect (110). It is widely established that IGF-1 has a major role in prenatal growth, independent of GH (111). During foetal life, the concentration of IGF-1 is positively correlated with gestational age (30-50% of adult levels), and a gradual increase occurs postnatally during childhood, peaking during pubertal development to achieve 2–3 times the normal adult values followed by a gradual decline with age (111,112). So far, numerous animals models and cell cultures studies have been attempted to distinguish between endocrine (circulatory IGF-1) and autocrine/paracrine (local IGF-1) effects in bone growth and metabolism (113). These studies clearly indicated that circulatory IGF-1 plays an important role in bone modelling (increasing bone formation), periosteal expansion, but a lesser role in longitudinal growth. In contrast, locally expressed IGF-1 plays a more important role in linear growth and bone metabolism through regulation chondrocyte differentiation, and coordination endosteal bone formation and resorption during growth and trabecular bone mineralization along with GH (113-115). However, it was suggested that circulatory and local IGF-1 appear to be redundant and can compensate for each other (116).

In addition to GH, IGF-1 signalling is required for PTH anabolic effects on bone (117). IGF-1 also has important insulin-like metabolic effects on peripheral tissues and plays a role in maintaining glucose homeostasis and insulin sensitivity (118).

1.2.5 GH Actions

GH has numerous biological actions, many occurring directly through the GH-R and indirectly through IGF-1. GH/IGF-1 has no specific target organ and it acts on most, if not all, tissues (Figure 1-5).

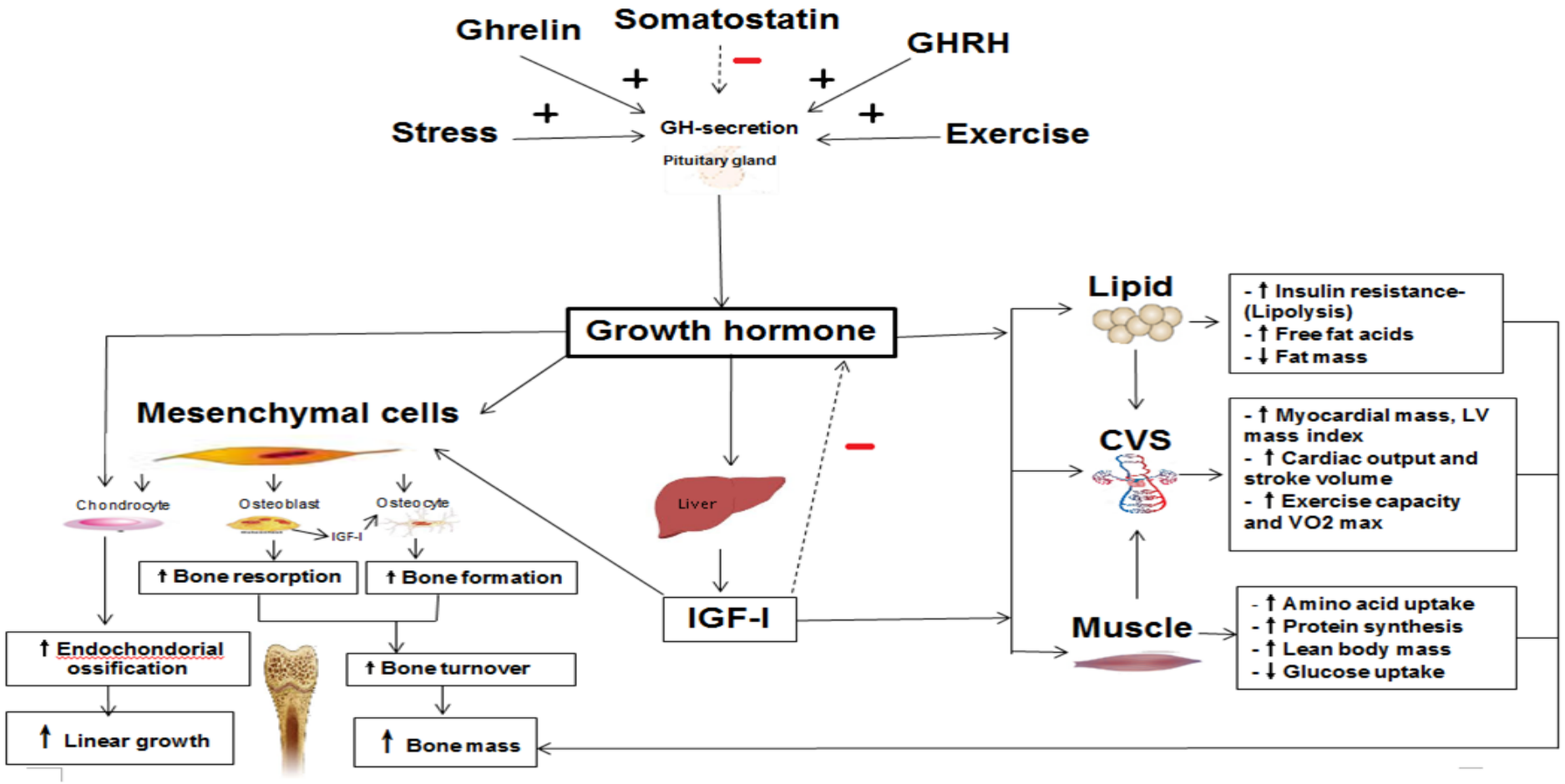


Figure 1-5 GH/IGF-1 axis and actions in bone, muscle and body metabolism.

+, stimulation: -, inhibition: GHRH, Growth-hormone-releasing hormone: CVS, cardiovascular system, VO2 Max: maximal oxygen consumption

1.2.5.1 Growth and bone

Both GH and IGF-1 have been shown to stimulate longitudinal bone growth make it difficult to attribute specific actions directly to GH or through IGF-1 (44,106). According to somatomedin hypothesis (Figure 1-6), GH can stimulate linear bone growth via systemic and local IGF-1 production (108). Evidence from animal models studies have clearly shown that bone growth is more severely reduced in the double null GH/IGF-1 than in either GH and IGF-1 null mice alone (119) and null GH mice showed a greater reduction in growth compared with only null IGF-1 (120). Recently, a study clearly showed that GH enhances linear growth without accompanying increase in IGF-1 levels (121), suggesting GH may have an IGF-1-independent effect on bone growth. On the other hand, it is also well documented that IGF-1 can stimulate bone growth in the lack of GH as in cases with GH receptor defects (Laron syndrome) (122).

In addition to severe growth retardation, null GHR mice showed reductions in cortical bone geometry and trabecular bone volume, and cross sectional bone area (123). Likewise, GH increase bone remodelling and turnover rate with a balance between bone resorption and formation either directly by interaction with GH receptors on osteoblasts or through producing IGF-1 (124).

GH directs stimulates the proliferation of osteoblasts lineage cells toward the osteoblastic and chondrocytic lineages over the adipocytic lineage. GH stimulates, either directly or indirectly via IGF-1, function of the differentiated mature osteoblast and bone formation (125). GH also stimulates the carboxylation of osteocalcin, which is a marker of osteoblastic activity. Unlike bone formation, the effects of GH and IGF-1 on bone resorption are less clear. GH and IGF-1 can stimulate osteoblasts to produced paracrine mediators and cytokines such as TNF-α and IL-6, which can promote osteoclastogenesis and osteoclastic resorption (126). It has been suggested that GH independently induces-osteoclast differentiation, whereas the activation of osteoclast is dependent on IGF-1 (106). Another mechanism has been suggested from in vivo and in virto studies that the GH and IGF-1 may influence the activation of osteoclast through alteration the balance of RANKL/OPG ratio (127).

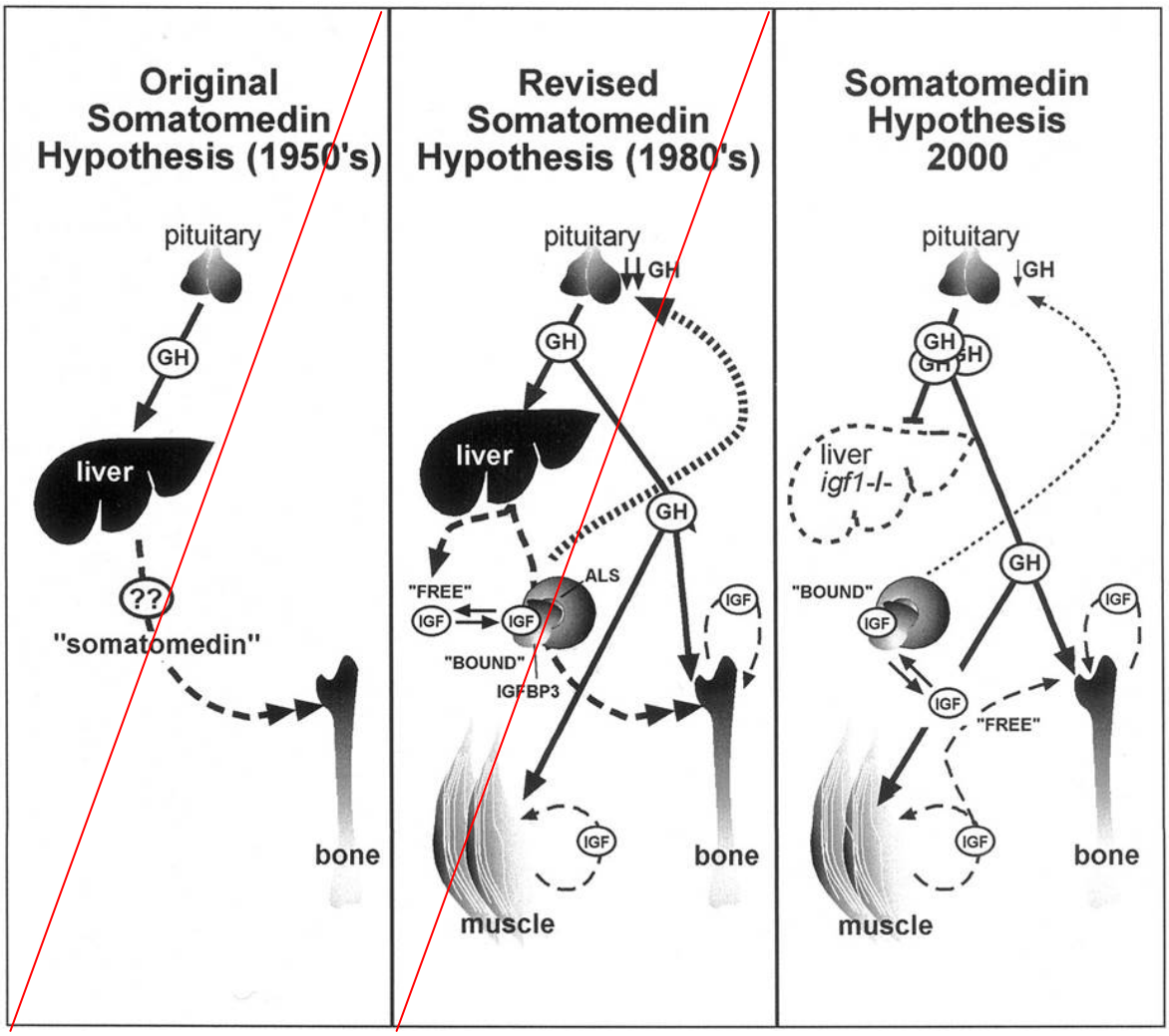


Figure 1-6 Somatomedin hypothesis.
Adapted and modified from (Le Roith et al. (108), Endocrine Rev, 2001).

In the 1950s, the original somatomedin hypothesis demonstrating that the pituitary gland produced somatomedian (ST), which, in turn, increased growth. In 1980s the somatomedian hypothesis was revised to put forward that growth is determined by GH acting primarily on the liver, where it stimulates IGF-1 synthesis and release. IGF-I then circulates to the main target organs, such as cartilage and bones, and thus acts in an endocrine mode. In 2000, the somatomedin hypothesis was revised again as the locally produced IGF-1—that is, synthesized locally in target tissues, but not the IGF-1 in the circulation—mediated the effects of ST in an autocrine or paracrine manner.

1.2.5.2 Muscles growth and metabolism

Both GH and IGF-1 regulate muscle metabolism by promoting positive protein balance by increasing protein synthesis and possibly through inhibiting protein breakdown by up-regulation of Lipoprotein lipase expression (LPL) (110). In addition, it has been reported that GH induces muscle hypertrophy which is likely mediated mainly by locally produced IGF-1 autocrine/paracrine actions, as IGF-1 appears to regulate human myotube size by activating protein synthesis, inhibiting protein degradation and inducing fusion of the reserve cells required to maximize growth (128). GH has also been shown to induce lipid accumulation in the muscles to shift in substrate utilization from glucose to lipids in the skeletal muscle which was well described in patients with excessive GH (acromegaly) (129). There is also insufficient evidence, mainly from theoretical animal studies, suggesting that GH may play a role in regulation of skeletal muscle fibre composition and induce a shift in muscle fibre from fibre type II (glycolytic fast-twitch fibres) to fibre type I (oxidative slow-twitch fibres) which may reflect the impact of GH on muscle strength and power (128,130).

1.2.5.3 Protein metabolism

GH has an anabolic effect on protein metabolism, as it stimulates protein synthesis while repressing proteolysis either directly or via IGF-1 endocrine and paracrine mechanisms (131,132). The majority of studies suggest GH has modest anabolic actions that may include increased protein synthesis and decreased breakdown at the whole body level, and decrease amino acid degradation/oxidation in muscles (133).

1.2.5.4 Lipid metabolism

GH has remarkable independent effects on lipids metabolism with little influence on IGF-1 through stimulation of lipolysis and ketogenesis. GH decreases body fat by increasing the hydrolysis of triglycerides(TG), releasing free fatty acids (FFA) and glycerol with increased lipid oxidation while decreasing FFA re-esterification(133,134). Data suggest that GH increases lipolysis by increasing adipose tissue hormone-sensitive lipase (HSL) and suppresses LPL activity mainly in visceral adipose tissue leading to reduced uptake of FFA from circulating very-low-density lipoprotein(VLDL) and TG (135,136). There is also some evidence to suggest that GH increases lipid metabolism by increasing the expression of low-density lipoprotein (LDL) receptors in the liver, enhancing LDL catabolism and inducing TG uptake by increasing LPL and hepatic lipase (HL) (135,137).

1.2.5.5 Glucose metabolism

GH is pivotal for the maintenance of glucose metabolism and homeostasis. Some of these effects are direct actions, whereas others are IGF-1 mediated largely through its insulin-like effects (opposite to those of GH) (133). GH lipolytic effect appears to be the most important monitoring of GH anti-insulin actions, through oxidation of FFA and subsequent inhibition of glycolytic enzymes, which ultimately inhibit insulin-stimulated glucose uptake (133,138). Furthermore, the GH anti-insulin effects included increasing hepatic glucose production and reduction in carbohydrate oxidation and hepatic and peripheral insulin sensitivity as mediated by its lipolytic effects (139). Other mechanisms have been implicated on the metabolic effects of GH through downregulation of insulin signaling included increasing suppressors of cytokine signalling (particular SOCS 1 and 3), and increasing expression of p85 regulatory sub-unit of PI3K activity in adipose tissue (135).

1.2.5.6 GH, energy expenditure and exercise

As described previously, GH is known as stimulates lipolysis and increases levels of FFA during resting and exercises. Data suggest GH effect on energy supply via stimulating ATP production from glycolysis, leading to an increase in anaerobic exercise capacity in skeletal muscle and enhance muscle function by increasing availability of FFA and pyruvate as metabolic fuels for energy production (128). GH also increases resting cardiac output and blood flow in several organs, including skeletal muscle and kidneys all of which are likely to elevate resting energy expenditure (140).

Exercise is one of physiologic conditions amplified secretion of GH, the GH peak of 10 ng/l was observed at 15-20 minutes after exercise test (141). Although the full mechanism of the influence of exercise on GH secretion is not fully understood, it is assumed that adrenergic mechanisms may play role as exercise induced GH may be enhanced by pre-treatment with beta receptor antagonist and inhibited by alpha receptor antagonist (141). It was also proposed that GH response to exercises which subsequently induced lipolysis and decrease abdominal fat (142).

1.2.5.7 GH and adipokines

Adipokines are bioactive peptides secreted by white adipose tissue and act at both local (autocrine/paracrine) and systemic (endocrine) levels, modulating lipid and glucose metabolism, inflammation, reproduction, cardiovascular function and immune systems (143-145). Leptin, adiponectin and resistin are the most commonly assayed adipokines, produced mainly by adipocytes of white adipose tissue, but not exclusively. There are other various products of adipose tissue including certain cytokines, such as tumour-necrosis factor (TNF), interleukin-6 (IL-6) and mediators that contribute to local and systemic actions (146). Leptin is a hormone product of the OB gene that regulates energy expenditure and food intake balance. Circulating leptin levels are

influenced by sex hormones, inflammatory cytokines and body fat (147). Leptin is involved in regulating energy balance through central actions, which is of importance particularly in the context of fat accumulation and metabolic disorders (148). Leptin also has many peripheral actions, mainly on the circulatory and respiratory systems, glucose homeostasis and reproduction (149). Adiponectin is a protein secreted exclusively by adipocytes and is the most abundant adipokine secreted by adipose tissue and expressed by bone marrow adipocytes. Adiponectin is involved in glucose synthesis in the liver, increasing insulin sensitivity, enhancing glucose uptake and fatty acid β-oxidation while decreasing gluconeogenesis in the skeletal muscle and liver, as well as having anti-inflammatory properties (150). Resistin belongs to the cysteine-rich family, and was discovered in 2001 in mouse adipocytes. Human resistin is produced and secreted mainly by peripheral-blood mononuclear cells (147). The physiological function of resistin in the mouse and human is still controversial. Data have shown that resistin may have a role in insulin resistance and diabetes in a variety of biological processes, including atherosclerosis and cardiovascular disease (151). It was suggested that GH may act as an important modulator in the production of adipokines, although the net directionality of these effects cannot be concluded (152). Data on the relationship between adipokines and the alteration of GH action show that GH lowers serum leptin levels, while its effects on adiponectin are contradictory in humans and rodents, with few studies on resistin (135). Recently, researchers have focused on the relationship between adipokines and bone metabolism (153). The critical role of adipokines and bone is highlighted by both adipocytes and osteoblast/osteoclast differentiation originating from mesenchymal cells, in which GH directs mesenchymal stem cells to adopt osteoblastic and chondrocytic lineages instead of adipocytic lineage, (Figure 1-7) (154,155). Leptin is known to play a role as regulator of bone metabolism by inhibits bone formation through decreasing osteoclacine and osteoclast inhibitor osteoprotegerin (OPG) (156,157). Adiponectin increases bone formation by stimulation of osteoblastogenesis, inhibiting osteoclastogenesis, and decreasing osteoclast numbers (158,159). Resistin has been shown to modestly increase the proliferation of osteoblasts in both cell and organ culture systems and increases the formation and activity of osteoclasts (160). Table 1-5 summaries the actions and the effects of leptin, adiponectin and resistin.

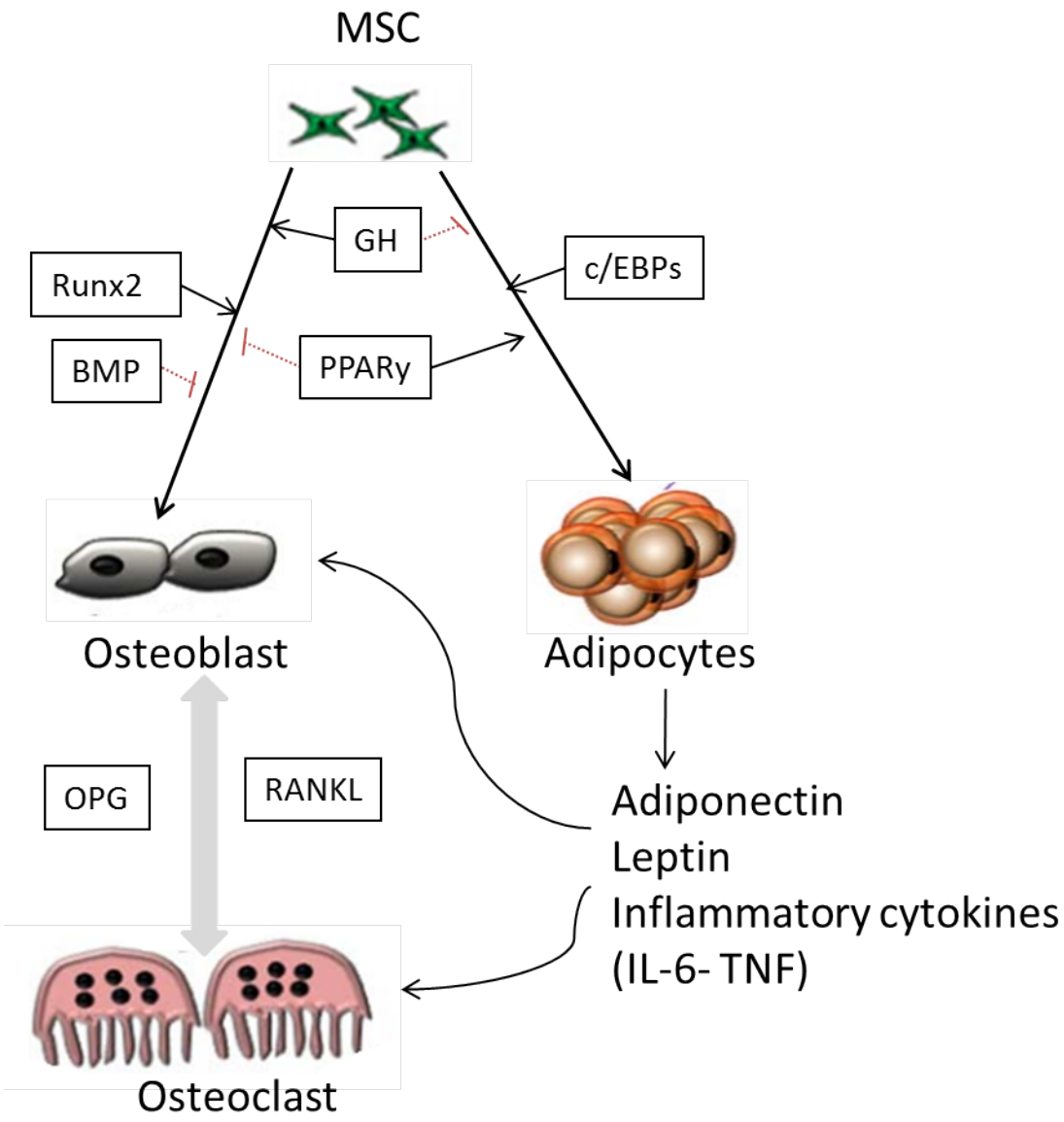


Figure 1-7 Schematic representation of mesenchymal stem cells (MSCs) differentiating into osteoblasts or adipocytes.

MSCs' commitment and differentiation toward either the osteoblast or adipocyte lineage is regulated by numerous pathways that converge on the regulation of four main transcription factors: peroxisome proliferator-activated receptor-γ (PPARγ), Runt-related transcription factor 2 (Runx2), bone morphogenic protein (BMP) and CCAAT/enhancer-binding protein(C/EBPs). Adipocytes blocks aosteoblastogenesis and promote osteoclastogenesis through PPARγ signalling and secretion of adipokines. GH blocks adipocyte lineage and stimulates osteoblast lineage.

Abbreviations: GH, growth hormone; MSCs, Mesenchymal stem cells; BMP, Bone Morphogenetic Protein; Runx2, Runt-related transcription factor 2; PPARy2, Peroxisome proliferator-activated receptor gamma 2;C/EBPs, CCAAT/enhancer-binding protein; RANKL, receptor activator of nuclear factor kappa B ligand; OPG, osteoprotegerin; Black sold arrow, stimulation; red dote arrow, inhibition.

Table 1-5 Adipokines, their action on bone and relationship to GH

Adipokines	Original	Metabolic	Bone	Relationship to GH	Ref
Leptin	A 16 kD a protein secreted by white adipose tissue cells , endothelial cells	- Regulates appetite and weight. - Mediator of immune-mediated diseases and has pro inflammatory effects	-It may have a regulatory role in osteoblast proliferation and differentiation - It inhibits bone formation through decreasing osteoclacine and osteoclast inhibitor osteoprotegerin (OPG) -It has both positive and negative effects on bone mass	-GH decreases leptin -Leptin may act as a metabolic signal to regulate GH secretion.	(152,156,157,161)
Adiponectin	A 28 kD a protein secreted by white adipose tissue cells, fibroblasts	- Mediator in the regulation of insulin resistance and has anti-inflammatory effects -Play role in pathophysiology of atherosclerosis	- Promotes bone regeneration by affecting the differentiation of MSCs to pre-osteoblasts - increases bone formation by inhibition of Osteoclastogenesis - Decreases osteoclast numbers and stimulation of osteoblastogenesis -Increasing mineralisation activity of osteoblasts proliferation and maturation.	No consistent relationship between GH and adiponectin either decreasing or no relationship	(152,158,159,161)
Resistin	A 12.5 kD a cysteine-rich polypeptide expressed and secreted by white adipose tissue ,monocytes, macrophages	- Induced endothelial cell activation, inhibits adipogenesis - Associated with insulin resistance and has pro-inflammatory properties	- Modestly increases the proliferation of osteoblasts and increases the formation and activities of osteoclasts in vivo studies	GH increases resistin	(152,160-162)

1.2.5.8 GH, bone mineral homeostasis and kidneys

GH and IGF-1 play an important role in adapting phosphate-calcium homeostasis to compensate rapid bone accrual and formation demands during growth in childhood and adolescence (163). GH increases renal phosphate retention by the stimulation of the maximum rate of renal tubular reabsorption of phosphate independent of PTH and vitamin D actions (164). GH mediates the action of IGF-1 on calcium homeostasis mainly through stimulation of 1 α-hydroxylase in the proximal tubule and the effect on vitamin D metabolism (164). The relationship between GH and PTH is still controversial, with studies suggesting that GH may have a regulatory role in PTH circadian rhythm (165).

Both GH and IGF-1 are known to cause sodium (Na) and water retention, although the exact mechanism underlying this antinatriuretic action is not fully explained. It was suggested that the possible mechanism may be related to GH stimulating Na+K+-ATPase activity in the distal nephron allowing for increased extracellular water (166). Other studies have suggested that GH may affect both renal haemodynamics and renal tubular function through direct stimulation of the renin-angiotensin-aldosterone system (RAAS) (167,168).

1.2.5.9 GH and quality of life

GH may affect cognition and psychology and thereby quality of life (QoL) by altering mental functions at central nervous system (CNS) sites. Emerging data from animal and human studies indicate that the GH/IGF-1 axis affects cognitive function and modulates the mood by modifying neurotransmission (dopamine - aspartate) (169,170). The evidence for this was based on a high density of GH and IGF-I receptors in brain areas known to be of importance in cognitive functioning, mood, memory, learning and sleep (171,172). There is also reliable evidence from clinical studies which revealed that patients with GHD showed cognitive impairment mainly in memory and attentional functions (173,174). In addition, GH may affect QoL via its effects on bone, body composition, cardiovascular function, and metabolism (175).

1.2.6 Therapeutic use

Human GH was first used in the late 1950s almost exclusively for children with clinical symptoms and short stature suggestive of severe GHD. After 1985, the production of biosynthetic GH using recombinant DNA techniques led to greater availability of recombinant human GH (rhGH) for all children with short stature without classical GHD criteria such as idiopathic short stature, growth failure associated with chronic renal insufficiency, growth failure in children born small for gestational age, and short stature in Prader-Willi syndrome, Turner’s syndrome, Noonan syndrome, and short stature home box-containing gene deficiency on the X chromosome (SHOX) (176). In general, children with these conditions appear to respond to pharmacological dosages of GH, although growth acceleration generally is not as good as replacement therapy of GHD (177).

Contraindications to rhGH use include active malignancy, active proliferative or severe non-proliferative retinopathy, acute critical illness, children with Prader-Willi syndrome (PWS) who are severely obese or have severe respiratory impairment, children with closed epiphyses, and hypersensitivity to somatropin or excipients (178).

1.2.6.1 Short term and long term adverse effects

The most common short-term adverse effects of rhGH treatment include headache, muscular pain, prepubertal gynecomastia, arthralgia, oedema, benign intracranial hypertension, and slipped capital femoral epiphysis. Symptoms are usually transient and resolved upon reduction of hGH dosage or upon discontinuation of the hGH treatment (179). Although long-term studies after 30 years of rhGH treatment are scarce, the majority of data are favourable towards the safety profile for most paediatric rhGH indications (180). A recent systematic review by Bunderen et al.(181) showed weak evidence that rhGH replacement is associated with an increased risk of primary malignancies or tumour recurrence, development of type 1 or type 2 diabetes mellitus, and overall mortality.

1.3 Growth Hormone Deficiency

Growth hormone deficiency (GHD) is an endocrine condition resulting from impairment of GH secretion or actions which can potentially impact on an individual's life from childhood, adolescence to young adulthood and later. In the UK, the prevalence of congenital childhood onset-GHD (CO-GHD) has been estimated to be between 1 in 3,500 – 4,000 live births, whereas the prevalence of adult onset (AO) GHD in addition to those with previous CO-GHD is as high as 3 in 10,000 of the UK adult population (178,182).

1.3.1 Aetiology

GHD may occur by itself or in combination with one or more other pituitary hormone deficiencies. Although there are many known causes of GHD, either congenital or acquired (Table 1-6), most cases appear to have an idiopathic basis with normal hypothalamic pituitary axis and it is not known whether that can be attributed to unreliable stimulation tests or unrecognised genetic defects (183). Clinically it is important to rule out all other causes of GHD before referring to the aetiology of the condition as idiopathic.

1.3.1.1 Congenital GHD

Congenital GHD causes can be further subdivided into genetic defects and anatomical abnormalities (e.g. hypothalamic-pituitary stalk transection, optic nerve hypoplasia, and cranial anomalies including holoprosencephaly). The majority of congenital GHD causes are sporadic with around 5-30% having a familiar pattern indicating a genetic compound (183). There are several genetic defects that lead to GHD such as those involving GH-1 and GHRHR genes. Congenital GHD can be also caused by pituitary anatomy defects include pituitary hypoplasia, pituitary aplasia, and congenital absence of the pituitary gland. Although these conditions often have no identifiable aetiology, ongoing advances in understanding pituitary development have provided a genetic basis to account for pituitary anomalies such as mutation of HESX1, PROP1 and others (184). In addition to structural developmental abnormalities associated with genetic causes, GHD can occur in the siting of other cranial or midline defects such as holoprosencephaly, nasal encephalocele, single incisor and cleft lip and palate, prosencephaly, septo-optic dysplasia (SOD), and midline craniocerebral or midfacial abnormalities, can be associated with anomalies of the pituitary gland (185). Many of these are associated with some genetic abnormalities (183).

1.3.1.2 Acquired GHD

GHD can be acquired at any time of life. A wide range of destructive lesions affect the hypothalamo-pituitary axis, ranging from tumours to infection, vascular effects, infiltrative diseases, or damage secondary to trauma, surgery, or irradiation. GHD may develop in about 35% of cancer survivors after cranial irradiation (186), although this development depends on radiation dose, patient age, and the nature of the underlying deficit. It was reported that 58% of children who underwent cranial radiation in excess of 30 Gy will have GHD and a relatively large proportion will develop additional pituitary hormone deficits within five years of radiotherapy (187).

Table 1-6 Aetiology of growth hormone deficiency (GHD)
Summarised from(188)

	Aetiology
Congenital	
Associated with midline structural defects	Agenesis of the corpus callosum, Septo-optic dysplasia, Holoprosencephaly, Encephalocele Hydrocephalus, Cleft lip or palate, Single central incisor
Genetic mutations	GRHR receptor, Pituitary transcription factors Hesx1 (Rpx), Ptx2 (Pitx2, P-OTX2, Rieg) Lhx3 (Lim-3, P-LIM), Types Ia, Ib, II, and III inherited IGHD, Multiple GH family gene deletions, GH receptor mutation IGF-Iand IGF-I receptor mutation, Stat 5b mutations
Acquired	
Tumours/irradiation	Craniopharyngioma, germinoma, optic glioma, dysgerminoma, ependymoma, pituitary adenoma, meningioma, chordoma
Head trauma	TBI: birth brain trauma; after neurosurgery ;Subarachnoid hemorrhage (pituitary apoplexy, vascular causes)
Inflammatory	Meningitis, encephalitis, pituitary abscess, sarcoidosis, tuberculosis, autoimmune processes, lymphocytic hypophysitis
Infiltration	Langerhans cell histiocytosis Iron overload: hemochromatosis, thalassemia and diseases requiring chronic transfusions

1.3.2 Clinical presentation

The variability and age at presentation are highly influenced by the time of onset, severity and duration of GHD at the time the patient first presents. In the neonatal period, a presence of hypoglycemia and midline facial defects will suggest the possibility of hypopituitarism (189). Infants who born with congenital GHD may show normal length standard deviation at birth but deceleration of growth is reported in the first 6-12 months of life (190). Children with less severe deficiency present later in life with short stature, delayed bone age and reduced growth velocity after excluding other causes of poor growth. Among children presenting with short stature, approximately 10% have pathologic GHD (191). They may appear 'chubby' or have a 'cherubic' facial appearance with flat nasal bridge and midface hypoplasia. In addition, sparse/thin hair, delayed closure of the anterior fontanelle, delayed dentition and delayed puberty may be seen (189).

1.3.3 Assessment of GHD during childhood and adolescence

Investigation of GHD in paediatric practice is based on auxological and clinical assessment combined with biochemical tests and pituitary imaging (188). However, establishment of GHD diagnosis in children is challenging with marked variability and lack of consensus on standard guidelines. After the initial auxological assessment, biochemical GH stimulation tests with different stimuli are employed to discriminate idiopathic short stature children and GHD (192). However, these tests still limit reproducibility and accuracy for the influence of sex, body composition and pubertal stage (193,194). The cut-off used to define GHD is arbitrary and varies according to the type of stimulus, but a peak level above 7-10 µg/l (20 mU/l) generally indicates a normal response (183,194,195). Given the limitations of the GH stimulation tests, it is generally advised that at least two tests are used for diagnosing GHD in children in order to improve sensitivity and specificity (178). In addition, both insulin-like growth factors-1 (IGF-1) and the IGF binding protein-1 type 3 (IGFBP3) in blood with a cut-off value below -2 SD for age and sex have also been used to assist with the diagnosis of GHD during childhood. However, it is frequently reported that IGF-1 and IGFBP3 levels have low reproducibility for the influence of chronic diseases and nutritional status, so the utility of IGF-1 and IGFBP3 measurements are subject to limited sensitivity (196).

In cases of high likelihood of GHD, imaging of the hypothalamic pituitary region obtained by magnetic resonance imaging (MRI) can contribute significantly to determining the cause of GHD. Molecular study of the GH gene in some cases can also assist in establishing the diagnosis.
At attainment of final height and satisfactory linear growth, adolescents with CO-GHD are required to have a reassessment of their GH axis as not all will have GHD as adults (197-199). According to established criteria, published guidelines classify CO-GHD on the basis of the probability of

persistent GHD into high likelihood and low likelihood (200-202). With a low probability of persistent GHD, GH stimulation tests are considered to re-evaluate GH secretion taking into account appropriate cut-off limits with different assay measurements (203)(204). GH peak cut-offs during transition are arbitrary with studies using either a peak GH cut-off <6.1 µg/l (205), <5.6 µg/l (198) or <5.1 µg/l (206), using the insulin tolerance test (ITT) as an acceptable criterion for GH replacement in the transition phase. In CO-GHD with a high probability of persistent GHD, a low IGF-I level (< -2 SD for age and gender) after at least one month of GH therapy is considered to be sufficient for persistent GHD without additional stimulation testing (207). The process and schema of diagnosis and re-evaluation of CO-GHD patients is summarized in (Figure 1-8).

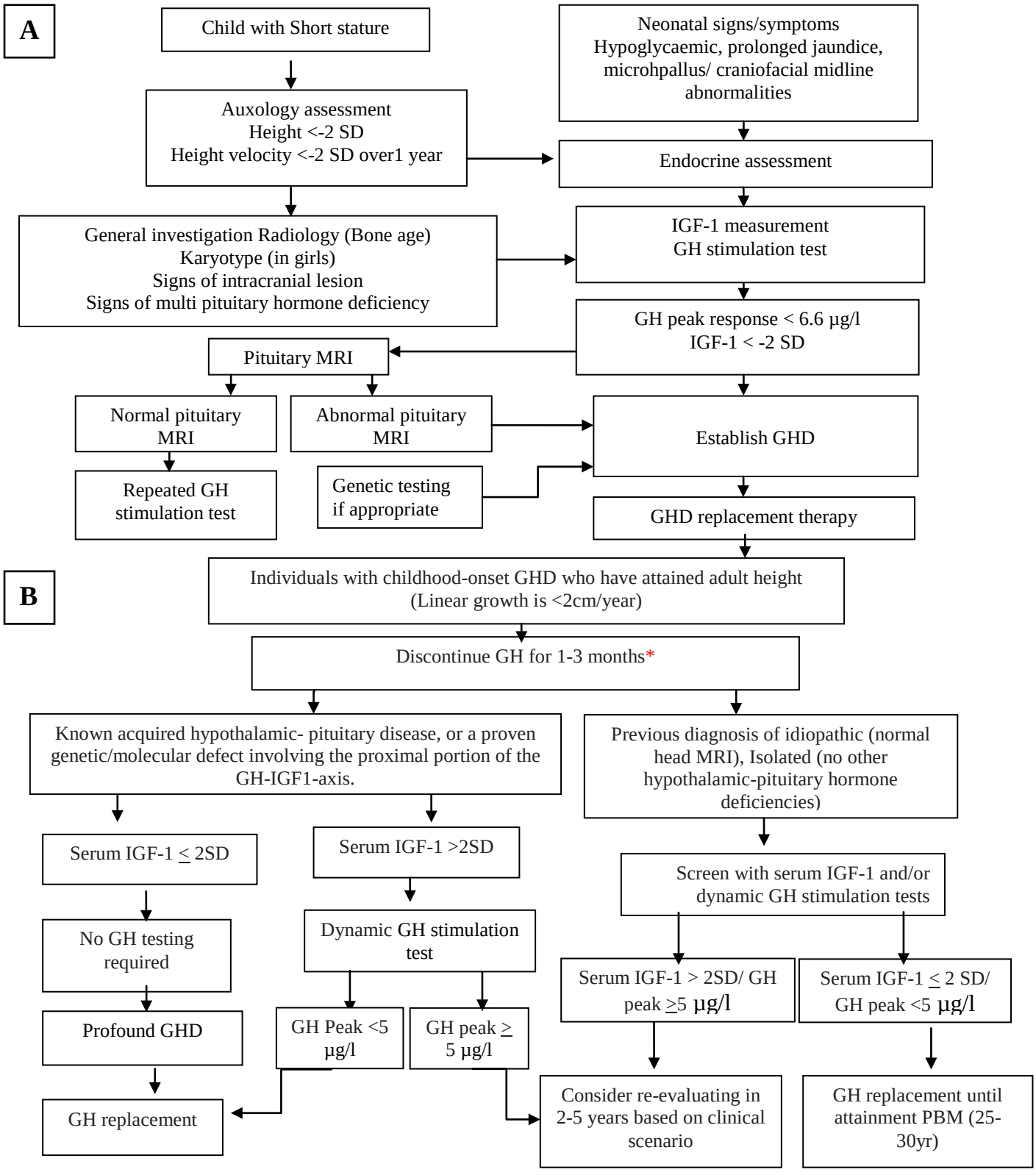


Figure 1-8 Schema for assessing (A) and reassessing (B) the GH/ IGF-1 axis during childhood and the transition period.
*patients with severe congenital or acquired panhypopituitarism with three or more pituitary hormone deficiency GH can be continued without interruption.

A schema is according to local practice; **B** schema is adapted and modified from Clayton et al. (200).

1.3.4 GH treatment, dose and monitoring

The doses of GH vary by age and by how it is calculated. In paediatric practice, a GH dose is usually calculated either according to body surface area [0.7–1.0 mg/m2/d] or body weight [0.17-0.35 mg/kg/wk] divided into daily subcutaneous injection. The total dose varies between Europe [25-35 µg/kg/d] and USA [25-43 µg/kg/d]. It is generally accepted that a daily dose of 25-35 µg/kg/d is sufficient to increase growth velocity to more than 10 cm/y in children with severe GHD (208).

GH dose is modified during transition towards adult treatment; 0.2-0.5 mg/d is recommended and not more than 2 mg/d (3 – 7 µg/kg/d in 70 kg individuals). Women generally require higher GH doses than men with 0.3 mg/d recommended for young females, 0.2 mg/d for young males, and 0.1 mg/d for older patients According to published recommendations, it has been suggested to start GH with a low dose (12.5 µg/kg/day), then titrate to attain IGF1 normal levels. IGF1 should be measured at 1- 2 month intervals during dose adjustment and at least once a year during therapy to be kept in a normal range appropriate for age and sex. Furthermore, the changes in body composition associated with GH treatment should be measured, such as DXA which is employed annually to measure lean mass (LM), fat mass (FM), and bone mineral density (BMD) (200-202).

1.3.5 Benefits of rhGH during childhood and transition

1.3.5.1 Childhood growth

In early-treated children, catch-up growth is excellent, with a normal final height and an average gain of 30 cm. The reported adult height in untreated GHD is between -6.1 and -4.8 SDS (134-146 cm) in males and -5.9 to -3.9 SDS (128-134 cm) in females (209), whereas treated GHD children grow at a mean velocity of 2.7 cm/year faster than untreated children and gain a mean adult height range between 1.5-2.0 SDS (210,211). However, this figure is affected by variables such as birth weight, height and age at the start of treatment, duration of treatment, frequency of growth hormone injections, and height at the start of puberty (212).

1.3.5.2 Benefits of rhGH during transition

The transition period, the time from mid to late teens until six or seven years after achievement of final height (200), is a critical phase for accrual of maximal peak bone mass and muscle strength, which is an important determinant of the risk of osteoporosis-related fractures in later life (51). During the transition period, healthy individuals' bone mineral density (BMD) and bone mineral content (BMC) increases 4-6 fold, together with lean mass (LM) over 2-3 years after attainment of final height (213-215). The effects of GHD and the benefits of rhGH therapy during transition have been reported in several studies as summarised in several reviews (216-219).

1.3.5.3 Bone mass and risk of fracture

1.3.5.3.1 Bone mass

GH plays a role in attaining and maintaining peak bone mass which is known to be a predictor of fracture risk and osteoporosis in late adulthood (220). For individuals with GHD, acquiring bone mass in childhood is influenced by several factors, including age of diagnosis, age at commencement and duration of rhGH treatment, height, weight, and body composition. Cross sectional and observational studies of bone density in rhGH treated children with CO-GHD at time of completing linear growth have shown inconsistent findings with either low areal bone mineral density (BMD) (g/cm2), normal or slightly reduced total body (TB) BMD, bone mineral density (BMC) and lumber spinal (LS) volumetric mineral apparent density (BMAD) (g/cm3) (221-225) (Table 1-7). Early rhGH treatment in childhood results in better indices of bone mass on completion of treatment at final height (221). Beyond transition, a longitudinal study reported a delayed timing of peak bone mass at LS and a rapid decline over the following 2 years was observed in adolescents with CO-GHD who discontinued rhGH after final height compared with controls (226).

Therefore, there was a concern that childhood rhGH treated subjects with CO-GHD may not achieve peak bone mass as a consequence of discontinuing GH treatment at final height.

Over the past few decades, a series of clinical trials studies have been conducted to examine the effects of continuation, discontinuation, and recommencement of rhGH during the transition phase of adolescents with CO-GHD, but thus far they yield conflicting results, Table 1-8. Continuation of rhGH is reported to be associated with an increase in TB-BMC and LS-BMD in the range 3-6% either after one year (227), or two years (228-230) as assessed with dual energy X-ray absorptiometry (DXA). However, this net gain is similar to what would be expected in the normal population during this stage (213,231).

It was also reported that bone mass does continue to increase in adolescents who discontinue rhGH therapy, yet the net gain is about half of that achieved by adolescents who continue rhGH therapy (228,229).

In contrast other studies have showed no change in BMD up to two years following discontinuation of rhGH after attainment of final height (223) , and no benefit from continuation of rhGH 2 years after final height (232,233). It was therefore proposed that 2 years was a safe period to be without rhGH, after which rhGH treatment would be recommenced in confirmed GHD patients. However, according to Tritos et al., an interval of 6-12 months off GH therapy was associated with a lower femoral neck (FN)-BMD and therefore a firm recommendation of a safe duration off rhGH replacement therapy with regard to BMD cannot be made (234).

Dose-dependency with regard to the impacts of rhGH on bone mass has only been studied in two studies; a 2-year randomized controlled trial found a higher dose (25 µg/kg/day) of rhGH impact differently in favour of bone mass than a lower dose (12.5 µg/kg/day) (229), when no significant difference was found in another similar study over same period (228).

It is noteworthy that among all the studies reported in table 2, there was considerable variability in definition of GHD during transition and retesting in terms of stimulated GH peak cut offs, population heterogeneity between isolated GHD/ multiple pituitary hormone deficiencies (MPHD) and aetiologies of GHD, duration of discontinuation of rhGH after final height, rhGH dose during childhood and after final height. In addition, measurement of bone density using DXA in children and adolescents with CO-GHD is challenging by confounding effects of body size and composition, with no consensus as to what is the optimal adjustment to express bone densitometry, additional to the lack of reference data that adjusts for different confounding factors of growth impaired children and adolescents (83). Therefore, this variation may substantially affect the interpretation of the results, limiting the analysis to certain groups of patients with the greatest benefit from the rhGH treatment during transition.

It is also increasingly recognised that the bone health and fracture prediction is not only dependent on bone density, but also on bone geometry and microarchitecture (82). The consequences of GHD and rhGH replacement on bone geometry and structure in subjects with CO-GHD have been investigated in few studies demonstrating reduced cortical area and thickness, but normal cortical and trabecular density at time of diagnosis during childhood which was significantly reduced after one year of rhGH treatment (235). At final height and after discontinuation of rhGH, marked lower height corrected cortical thickness and wider endosteal circumference, but a normal cortical and trabecular density compared with a healthy reference population (236). Two years of rhGH replacement results in a significant increase in cortical thickness compared to non -treated control group of young adults with CO-GHD (237), when a significant reductions in cortical bone area and thickness in untreated CO-GHD adults compared to AO-GHD was reported elsewhere (238). One recent study with more advanced imaging (high-resolution magnetic resonance imaging (micro-MRI) investigated the bone structure of ten young adults with hypogonadism and/or CO-GHD and reported that ratio of apparent bone volume to total volume (appBV/TV) and apparent trabecular number (appTbN) were significantly lower in GHD than in the age-matched control group (239), although the relationship between trabecular size and number, to bone fragility and fracture risk has not been established yet.

Table 1-7 Summary of cross sectional studies, non-interventional- observational studies of the effects of GHD adolescents with CO-GHD.

Ref	N	MPHD/ IGHD	Design	Age yrs	Tool	Groups	TB	LS	Body composition	CVS risks	Glucose metabolism	QOL
(240) (223) (241) (242)	40	28/12	2-yr-Long	16±21	DXA	n=22 GHD n=19 GH-sufficient n=16 control	↑5% -BMC	↑4% BMD	↓ 8% in LM in GHD ↑7% FM% in GHD	↑ in GHD	↔	↔[1]
(226)	16	0/16	6-yr-Long	17·1 ± 0·9	DXA	n=16 GHD	-	↓ areal and volume BMD	-	-	-	-
(236)	90		C.S		PQCT	n=37 GHD n=53 GH-sufficient	↓ cortical thickness Z-scores in both ↑ Cortical CSA in both ↓SSI Z-score in GHD	-	↓ Muscle CSA in GHD ↑fat/muscle in GHD	↓ HDL ↑LDL/ HDL	-	-
(225)	18		C.S	18-30	DXA isokinetic dynamometer	n=9 GHD n=9 GH-sufficient n=18 control	↓ BMD in GHD and GH-sufficient vs. Control	↓ BMD in GHD and GH-sufficient vs. Control	↓ LM ↓FM ↓ muscle strength in GHD and GH-sufficient vs. control	-	-	-

↑,increase ; ↓,decrease; ↔, no significant changes or different; Long, longitudinal; C.S, cross sectional; n, Number of patients; GHD, growth hormone deficiency; ; IGHD, Isolated growth hormone deficiency; MPHD, multiple pituitary hormone deficiencies; DXA, dual energy x-ray absorptiometry; PQCT, peripheral quantitative computed tomography; BMD, bone mineral density; BMAD, bone mineral apparent density ; BMC, bone mineral content; LM, lean mass; FM, fat mass; LS, lumbar spine; TB, total body; CVS, cardiovascular system; HDL, high-density lipoprotein ; LDL, low-density lipoprotein; [1] Nottingham Health Profile, Psychological General Well-Being, Mood Adjective Check List, visual analog scale and more

Table 1-8 Summary of RCT and longitudinal studies of the effects of GHD and rhGH replacement in adolescents with CO-GHD

Ref	N	MPHD/ IGHD	Age yrs	Design	Groups	rhGH doses	TB	LS	Body composition	CVS risks	Glucose metabolism	QOL
(243) (244)	18	15/3	20.2+1	2-yr RCT	n= 9 on rhGH n=10 on placebo	3.6 IU/d	-	-	↑6% in LM in rhGH ↓ 6% FM in rhGH	↔	↓IS in rhGH	↔[2]
(227) (245)	24	20/4	17±1.4	1 yr. RCT	n=12 on rhGH n=12 no rhGH	17 µg/kg/d	↑6% -BMC in rhGH	↑5% BMC in rhGH	↑ (6%) LM in rhGH No change FM	↔		-
(229)	64	52/12	23±4.2	2 yrs RCT	n=20 on adult GH n=23on paed GH n= 21 Placebo	12.5 and 25.0 µg/kg/d	↑3.3 % BMD adult GH ↑5% BMD in paed-GH ↑1.3 % BMD placebo	-	↑LM of 13.4% in rhGH Vs 3.1% in placebo	↔	-	↔[3]
(228) (246) (247)	92	72/20	19±2.8	2-yr RCT	n=59 on adult -GH n=58 on paed-GH n=32 on Placebo	12.5 and 25.0 µg/kg/d	↑ 9% BMC in rhGH ↑5% BMC in placebo ↑5% BMD in rhGH ↑ 3% BMD in placebo	-	↑14% LM in rhGH Vs 2% in no GH ↓ FM	↔	-	↔[4]
(232)	58	25/33	15.8	2 yrs RCT	n=25 on rhGH n=15 on placebo n=18 GH-sufficient	20 µg/kg/d	↔ in BMD across all groups at baseline and after 2y	↔	↔ in LM ↔ in FM	↔	↔ HOMA-IR- QUICKI	↔[3]
(248)	10	5/5	17–20	1yr Long	n= 10 on rhGH n=10 control	8–10 µg/kg·d	-	-	-	+ effect on lipids ↔in IMT in GHD ↓IMT in GH-sufficient	↑ HOMA in rhGH ↓HOMA in GH-sufficient	-
(249)	23	9/14	15-20		n=15 on rhGH n=8 GH-sufficient n=23 control							
(230) (237)	160	35/125	18-25	2yrs, RCT	n=109 on rhGH n=51 no rhGH	0.2-0.4 mg/d	↔in BMD ↑cortical thickness ↓endosteal diameter	↑3.5% BMD in rhGH	-	-	-	-
(233)	40	12/28	15.6-17.3	2ys Long	n=23 on rhGH n=17 no rhGH	0.4–1.3 mg/d	↔BMD SDS	↔ BMAD	↓LM ↑FM in untreated	-	-	-

↑,increase ; ↓,decrease; ↔, no significant changes or different; Long, longitudinal; RCT, randomised control trial; n, Number of patients; rhGH, recombinant human growth hormone; BMD, bone mineral density; BMAD, bone mineral apparent density ; BMC, bone mineral content; LM, lean mass; FM, fat mass; LS, lumbar spine; TB, total body; CO, childhood-onset GH deficiency; IGHD, Isolated growth hormone deficiency; MPHD, multiple pituitary hormone deficiencies; CVS, cardiovascular system; IMT, intima-media thickness; HOMA-IR, Homeostasis Model Assessment- Insulin resistance; IS, insulin sensitivity; QUICKI, quantitative insulin sensitivity check index.1 Nottingham Health Profile, Psychological General Well-Being, Mood Adjective Check List, visual analog scale and more; 2 General Health Questionnaires (GHQ); 3AGHDA; 4 QLS-H questionnaires

1.3.5.3.2 Risk of fracture

Although data on the association between bone density and fractures in children is limited, it is generally established that the fracture risk may be higher in healthy children and adolescents who have low BMC and bone accrual (250,251). The association between GHD, low bone mass and subsequent fracture risk in adolescents and young adults with CO-GHD is less clear than that observed in adults with GHD and hypopituitarism (252,253). However, in these studies, it was not known if that is a result of being GH deficient *per se* or due to other pituitary hormone deficiencies. Accordingly, other studies showed no evidence that isolated GHD (IGHD) may increase fracture risk (254,255). With regard to the impact of rhGH replacement therapy on fracture rates, childhood studies suggest a protective effect of rhGH treatment in children with GHD with a fourfold decrease in fracture frequency from diagnosis to final height compared to matched healthy controls, but fracture prevalence increased to 3 % at final height particularly in those with reduced lumbar BMD (Z-score <1) (224). Studies in adults involving both CO- and AO-GHD reported a lower incidence of fracture risk in CO-GHD compared to AO (252,254,256), with a double incidence of non-osteoporotic fracture in women with CO-GHD compared to men with CO-GHD despite continuation of rhGH treatment (256), Table 1-9. In view of these studies, CO-GHD was queried as a cause of osteoporosis due to the lack of evidence for increased fracture risk in children and adults with CO-GHD or severe GH resistance (92).

To summarise this section, data thus far demonstrate contradictory results, with most studies, but not all, showing a small increase in bone density and mineralisation during rhGH therapy in transition. However, the extent of GHD and replacement with regard to bone density and architecture is unclear. Using more advanced non-invasive imaging tools that assess bone quality, may provide a greater insight into the effects of GHD and rhGH on bone.

In addition, there is insufficient evidence of increased fracture risk in patients with CO-GHD as the reporting of the risk of fracture in GHD had considerable limitations. Therefore, it remains unclear whether early adulthood rhGH treatment would offer protection from osteoporosis and fracture risk in late adulthood. Prospective long-term follow up studies are still lacking.

Table 1-9 GHD and fracture risk in young adults with CO-GHD

Ref	Design	CO/AO	MPHD/ IGHD	Age (yrs)	Duration of rhGH (yr)	Measurement of outcome	Result	Fracture sites	Comment
(224)	Cross sectional	46/0	0/46	14.8–19.9	8.6 ± 1.6	Prevalence of fracture	No different vs normal population	Osteoporotic fractures*	LS BMDvolume of fractured patients was significantly lower than fracture-free
(255)	Cross sectional	66/0	27-OMPHD 21-CMPHD 18-IGHD	≥18 yr.	n=43 never received GH	Lifetime low-energy fracture prevalence	IGHD no risk OMPHD OR = 3.0; 0.6 CMPHD OR=7.4; 2.2 fractures per patient	All sites, more at wrist,	TB,LS,FN-BMC ,areal BMD, and volumetric BMD were marked decreased in all group more in OMPHD
(254)	Cross sectional (KIMS)	709/ 2159	602/107	23-28	One year	Prevalence of fracture risk	20% in CO-GHD vs. 25% in AO	-	No bone density data
(256)	Cross sectional	100/732	68/32	27-28	12-15	Fracture incidence rate ratio	Women COGHD double increase IRR(2.3) No change in IRR of CO GHD men (0.6)and AO (0.5)	Non osteoporotic fractures	No bone density data

OR, odd ratio; IRR, incidence rate ratio; COGHD, childhood-onset GH deficiency; AO, adult-onset GHD; IGHD, Isolated GHD; MPHD, multiple pituitary hormone deficiencies; OMPHD, open growth plates MPHD;CMPHD, close growth plates MPHD; LS, lumbar spine;TB, total body; BMD, bone mineral density; KIMS, the Pharmacia& Upjohn International Metabolic Database

* Osteoporotic fractures = vertebra, wrist, upper arm, and hip

1.3.5.4 Body composition and muscle strength

During transition, studies indicate that patients who were reconfirmed to have persistent GHD and discontinued rhGH in the transition period showed decreased lean mass (LM) (-8%) and increased fat mass (FM) (10-17%) compared to either sufficient or those who continued rhGH after 2 years of observation (233,240,243,245). A study measured the early changes in body composition in CO-GHD patients after a median of 6 months after cessation of rhGH in patients who attained final height. The authors stated that patients with persistent GHD (n=37) had a significantly lower muscle cross-sectional area (CSA) Z-score (-0.24$\pm$1.6 vs. 0.44$\pm$1.42, $p<0.03$), a 2 fold increase in fat CSA (1329+ 100 mm^2 vs. 878+91mm^2) compared to patients who were no longer GH deficient at final height (236). Recommencement of rhGH therapy was documented to result in a marked improvement in body composition, with an increase in LM by 14%, and reduction in FM by -7% over two years of replacement (229,246), yet longer term studies are scarce in determining the sustainability of these changes. Mauras et al. is the only study that showed no significant difference in the changes of LM and FM from baseline to two year between continuation of rhGH as compared to placebo-treated or control subjects (232).

In terms of the relationship between CO-GHD, rhGH and muscle strength, it has been reported that discontinuation of rhGH in CO-GHD for two years has potentially negative consequences on muscular strength in some studies (241,257), but not all (232,243). From a recently published cross sectional study investigating muscle strength and body composition of 18 males with CO-GHD (aged 18-30 years), of those, 9 (4-IGHD) were reconfirmed to have GHD after re-evaluating them at final height during transition. This study suggested muscle strength as measured by a isokinetic dynamometer was lower in those with persistent GHD compared to sufficient and healthy controls ($p<0.05$) (225). However, data so far do not support the use of rhGH therapy to increase muscle strength during transition and young adulthood (229,233,243).

The majority of research has shown favourable differences in body composition with recommencing rhGH during transition, although encouraging, further research in the field with long-term follow-up is needed.

1.3.5.5 Cardiovascular risks

Epidemiological evidence shows negative effects of GHD on cardiovascular risk factors including unfavourable lipid profiles, hypercoagulability, atherosclerosis and endothelial dysfunction, which could contribute to increased morbidity and mortality of adults with GHD and hypopituitarism without rhGH therapy (258), with a higher hazard ratio in AO- compared to CO-GHD (3.0 (2.1–4.4) vs. 1.4 (1.0–1.8) respectively (259). Cardiovascular risk in CO-GHD and benefits of rhGH have been documented during childhood (260,261) and adolescence (248).

1.3.5.5.1 Lipid profiles

It has been well-established that discontinuation of rhGH therapy after final height results in an increase in unfavourable lipid profile (236,246,249,262), while the effect of restarting rhGH therapy remains unclear. Some studies have shown reversal in the levels of unfavourable lipid profiles (263), whereas others report no change in lipid profile either on cessation or continuation of rhGH therapy during transition (229,232,245). A study of KIMS database (Pfizer International Metabolic Database) reported that those who were older at first starting childhood rhGH (short duration of childhood rhGH replacement) and had a longer time off rhGH during transition were more likely to have higher total cholesterol and triglyceride levels during transition (264).

1.3.5.5.2 Cardiac structure and performance

At final height, cross sectional echocardiographic studies indicate that all cardiac dimensions of adolescents with GHD who were treated with rhGH during childhood were significantly smaller than their age-and sex- matched healthy controls after withdrawal of rhGH (5.7$\pm$4.5 years), whereas reinstituting rhGH results in a significant increase in LV mass and LV mass index after 16-24 months (265) with improvement in endothelial function within the first 6 months of restarting rhGH (266).

There is also conflicting data on alterations in carotid artery intima-media thickness (IMT), a surrogate marker of early atherosclerosis with increasing in IMT thickness, in subjects with CO-GHD. Murata et al. showed a significantly higher IMT in adults with CO-GHD compared to both adults with AO-GHD and healthy controls (267). However, this alteration in IMT was not evident in adolescents with CO-GHD during and after discontinuation of rhGH (249,268). A study involved 23 subjects with CO-GHD (14- IGHD) (aged 15–20 years) showed that 6 months off rhGH in adolescents who were confirmed GHD did not result in a significant alteration of the common carotid arteries, whereas in adolescents who were not confirmed to have GHD, IMT increased during rhGH treatment and reversed to normal 12 months after rhGH withdrawal (249).

In summary: the current evidence suggests that discontinuation rhGH during transition is associated with a pro-atherogenic lipid profile; however, the effects of recommencement of rhGH treatment and a prolonged period off treatment are less clear. There is no evidence demonstrating that

discontinuation of rhGH therapy during transition has any detrimental consequences on the cardiovascular system in the short or long term.

1.3.5.5.3 Glucose metabolism

Few studies have investigated CO-GHD and its replacement on insulin and glucose metabolism during transition in relation to concomitant changes in body composition and metabolism.
After cessation of rhGH at final height, some studies reported an increase in; insulin sensitivity as estimated by either means of a hyperinsulinemic euglycemic clamp (244) or homeostasis model assessment (HOMA) (245) and increase in fasting glucose (243) in those who had persistent GHD, with similar changes were reported elsewhere in those who were not confirmed to be GH deficient at final height (269). Inversely, significantly impaired insulin resistance as measured by HOMA was recorded within 6 months off rhGH, but returned to baseline levels after 6 months after restarting rhGH replacement (249). At two years of resuming rhGH therapy during transition there was an insignificant or limited effect on insulin resistance, insulin sensitivity and glycosylated haemoglobin (HbA1c) (229,232,244). In addition to the variation of techniques used to assess glucose homeostasis in these studies, other factors particular body compositions and short term duration results in limited evidence with regards to impairment of glucose homeostasis in GHD and rhGH replacement during transition. Long-term studies are necessary to identify the influence of different aspects of GHD and replacement on glucose homeostasis during transition.
Generally, there is weak evidence that GHD or rhGH replacement induces an increase in the risk of type 2 diabetes (T2DM) in subjects with GHD. With regard to GHD, the KIMS database has demonstrated that the prevalence of T2DM in untreated adults with AO-GHD and hypopituitarism was higher than expected with an overall standardised prevalence proportion ratio (1.13 (95% CI, 1.04–1.23%)), which was largely to be explained by high BMI and the adverse body composition (270). In terms of rhGH replacement, there is an uncertain relationship between rhGH treatment and the risk of T2DM in particular in those with GHD, and whether rhGH therapy leads to increased risk of diabetes has not been established yet. Paediatric studies demonstrated modest increases in the incidence of T2DM in rhGH -treated children with predisposed risks relative to the general population, but not in those with GHD individually (271,272).
In conclusion, in GHD, there is insufficient evidence available to conclude whether or not rhGH therapy in childhood or transition alters insulin sensitivity and increases the risk of T2DM in adulthood. More research is needed to clarify the elements of the dual effects of GH during transition in adolescents with CO-GHD with regards to both the impact on body composition/BMI and insulin resistance.

1.3.5.6 Quality of life (QoL)

The health related quality of life issue has emerged as an important aspect in consideration of rhGH therapy in adulthood, but not during childhood or transition (273). In relation to QoL in individuals with CO-GHD, some studies reported that children and adolescents with GHD have some difficulties with psychosocial functioning, mood, behaviour and cognitive ability (274) despite the achievement of acceptable final height (275). A retrospective study suggested that adolescents with CO-GHD who were not treated with rhGH after attaining final height have some psychological difficulties with self-confidence and social contact, and this was worse in those who were either rhGH treated after the age of 12 years or those who were shorter at the start of treatment (274). A report from the KIMS database showed a positive relationship between height gain during childhood treatment and improvement in QoL at transition and an inverse relationship between QoL and duration off rhGH therapy with a longer period off rhGH associated with a poorer QoL (264). Re-instituting rhGH treatment has a significant positive change in health related QoL aspects (242,264). However, longitudinal studies evaluated the effects of discontinuation and resumption of rhGH treatment on QoL in young adults with CO-GHD, showed that discontinuation of rhGH treatment for one year leads to a decrease in QoL within 6 months, which is counteracted in 3-6 months after re-initiating rhGH therapy (276,277). This was disputed in follow up and RCT studies showing that QoL is less effected in adolescents with GHD measured after discontinuation rhGH at final height (242) with no difference in being off rhGH therapy and after re-commencing rhGH (229,232,247). However, using different questionnaire tools (generic and disease-specific questionnaires) which assess different dimensions of health related quality of life in adolescents with CO-GHD makes comparisons of the outcomes of these studies difficult.
In summary, there is variability in the assessment of QoL by different studies in terms of the instruments used and the effects measured which may reflect the different outcome results in QoL. In addition, QoL is multifactorial and factors such as short stature combined with other pituitary hormones deficient may influence QoL in this particular group of patients. To date there is no clear consensus on the appropriate QoL measurement tools in children and adolescents with GHD. Therefore, there is currently no evidence of reduced QoL that rhGH may have beneficial effects on QoL in subject with CO-GHD during transition.

1.3.5.7 Summary

GHD is an important condition that has detrimental effects on both physical and psychological health throughout life, whereas rhGH therapy shows benefits in both children and young adults with GHD throughout each stage of their life. It seems from the current data that rhGH has less direct impact on bone density, with a greater impact on body composition and cardiovascular risk factors, including improvement in serum lipid profiles, and to a lesser extent on insulin sensitivity and QoL. Even with scarce evidence, substantial short term studies during transition revealed untreated GHD has a risk of alteration in somatic and metabolic consequences, although it is difficult to establish whether these mild alterations represent the early long-term consequences and whether subsequent rhGH treatment improves long term health. Larger studies, of longer duration of rhGH therapy will be required to determine whether the metabolic alterations in adolescent GH-deficient patients persist in later adulthood and if recommencement of rhGH therapy has a positive impact on these aspects.

1.4 Weight Bearing Exercise and Bone Health

It has been well established for several decades that physical activity and exercise are associated with promotion of physical and psychological aspects of growth and development. Studies highlight the positive impact of exercises in management of several existing chronic illnesses and prevention of long term complications (278), with a strong emphasis on physical activities being a preventative measure to combat or offset osteoporosis and fractures (279). Substantial experimental and clinical evidence has highlighted the importance of functional loading for optimal bone gain and strength during growth and reduced bone loss later in life (280). Weight bearing exercise (WBE) in particular has been found to enhance bone health parameters during growth (281). WBE is defined as force-generating exercises placing higher mechanical stress on skeletal regions, such as jumping, aerobics, circuit training, volleyball and other sports that generate impact on the skeleton (282). Studies have shown that WBEs have a greater osteogenic effect on bone than non-weight-bearing exercises (283). High impact WBEs such as jumping exercises are studied extensively in relation to bone mass and bone mineral density. Jumping exercises provide a dynamic loading effect on the bones through the axial mechanical load elicited when ground-reaction forces reaches 6-8 times body weight (284). In the majority of studies, jumping exercise, even with low repetition, is efficient. Kato et al. (2006) reported that 10 maximal vertical jumps three times per week increased femoral neck BMD by 3.8% and lumbar spine BMD by 1.8% during six months of training in premenopausal women (285). A review of bone growth and exercise suggests childhood and adolescence is the optimal time that exercise programs can improve bone strength by maximising peak bone mass (286,287), and that declined precipitously with late adolescence (288). Importantly, the bone benefits from WBE seem to be maintained into adulthood and may reduce the facture risk in later life (289). For this reason, there has been considerable interest in quantifying the effects of exercise on bone accrual during growth and defining the appropriate mode, intensity, frequency and duration of exercise, in addition to the precise timing of exercise (childhood or adolescence), required to optimize bone health throughout life.

1.4.1 Mechanism of influence of weight-bearing exercise on bone

The link between WBE and positive gains in skeletal mass is illustrated by three main mechanisms as seen in Figure 1-9.

1.4.1.1 Exercise effects on bone through mechanical force

When mechanical force is applied over bone tissue, bone tissue is deformed at the site where loading acts. Osteocytes and bone-lining cells detect that bone is being loaded and generate hydrostatic pressures within bone canaliculi and interstitial spaces causing tissue fluid to move through the canalicular spaces. Fluid flow creates a fluid shear stress which leads to a transduction of the mechanical signal into a biochemical response of the effector cell (osteoblast-osteoclast) to initiate formation or resorption of bone cell tissue through an increase in the levels of the osteogenic factors such as intracellular calcium, prostaglandin E2, nitric oxide and others (290,291). These products are potent regulators of osteoblasts and osteoclasts during bone remodelling and stimulate new bone formation by promoting both proliferation and differentiation of osteoblastogenesis (292). The process by which mechanical forces are converted into biochemical responses that are then integrated into cellular responses is known as mechanotransduction. There are several factors that can have an effect on the mechanotransduction process in bone, such as type and frequency of mechanical loading, age and gender (293). Although osteocytes act as primary mechanosensors of mechanical loading on bone, mechanical loading also has an impact on osteoblasts leading to reduce recruitment and differentiation of osteoclasts by augmentation of OPG and reduction of RANKL expression, which in turn reduces the possibility of bone loss (294).

1.4.1.2 Effects of exercise on bone through muscle

Another mechanism of bone adaptation to mechanical loading has been suggested to be explained by the mechanostat theory (a refinement of Wolff's law), by which load bearing bones maintain shape and strength in response to muscle strain and mechanical usage (66). It has been suggested that muscle contraction activates bone mechanoreceptors in the bone periosteum (291). Another suggested mechanism is where exercise activates IGF-1 and insulin receptors in muscle in response to activated bone mechanoreceptors and these receptors play a paracrine role on periosteal cells and apoptosis inhibition (295). These theories support the evidence that peak rates of bone mineral acquisition are preceded by peak rates of muscle mass gain, strengthening the idea that exercise programs aiming first to improve muscle mass and strength would stimulate bone formation and acquisition of bone mass (296).

1.4.1.3 Effects of exercise on bone through hormones

Exercise has been found to affect the levels of calciotropic hormones, vitamin D and parathormone (PTH), which are essential regulators of bone metabolism (297). Several studies have shown that acute resistance exercise can increase concentrations of anabolic hormones, such as GH/IGF-1 and FSH/LH/oestrogen, across a wide age range (298). These anabolic hormones have been shown to have either positive or negative impacts on bone growth and metabolism under the influence of exercise intensity. as summarised by Chilibeck 2010 (299) in Table 1-10.

Table 1-10 Effects of exercise on bone through hormones.
Adapted and modified from reference (299)

Hormone	Effect of hormone on bone	Effect of exercise on hormone level
Oestrogen	↑ Ca absorption; ↓ Bone turnover	Extreme training with low energy intake: ↓ release
Progesterone	↑ Bone formation and resorption	Same as above
Testosterone	↑ Ca absorption; ↑ bone formation	Extreme training: ↓ release Acute exercise ↑ release Chronic exercise ↑ release or ↔
Growth hormone	↑ Bone formation; ↑ Production of active form of vitamin D	Acute exercise: ↑ release Chronic exercise: ↔
IGF-1	↑ Bone formation	Acute exercise: ↑ release Chronic exercise ↑ release, or ↔
PTH	↑ Bone resorption when continuously released ↑ Bone formation when intermittently released	Extreme training: ↓ release Acute exercise: ↑ release Chronic exercise: ↔
Calcitonin	↓ Bone resorption	Chronic exercise ↔ or ↓
Vitamin D	↑ Ca absorption	Extreme training: ↓ release Chronic exercise ↑ release

↑ = increase; ↓ = decrease; ↔ = no change; IGF-1 = insulin-like growth factor 1, PTH= parathyroid hormone, Ca = calcium

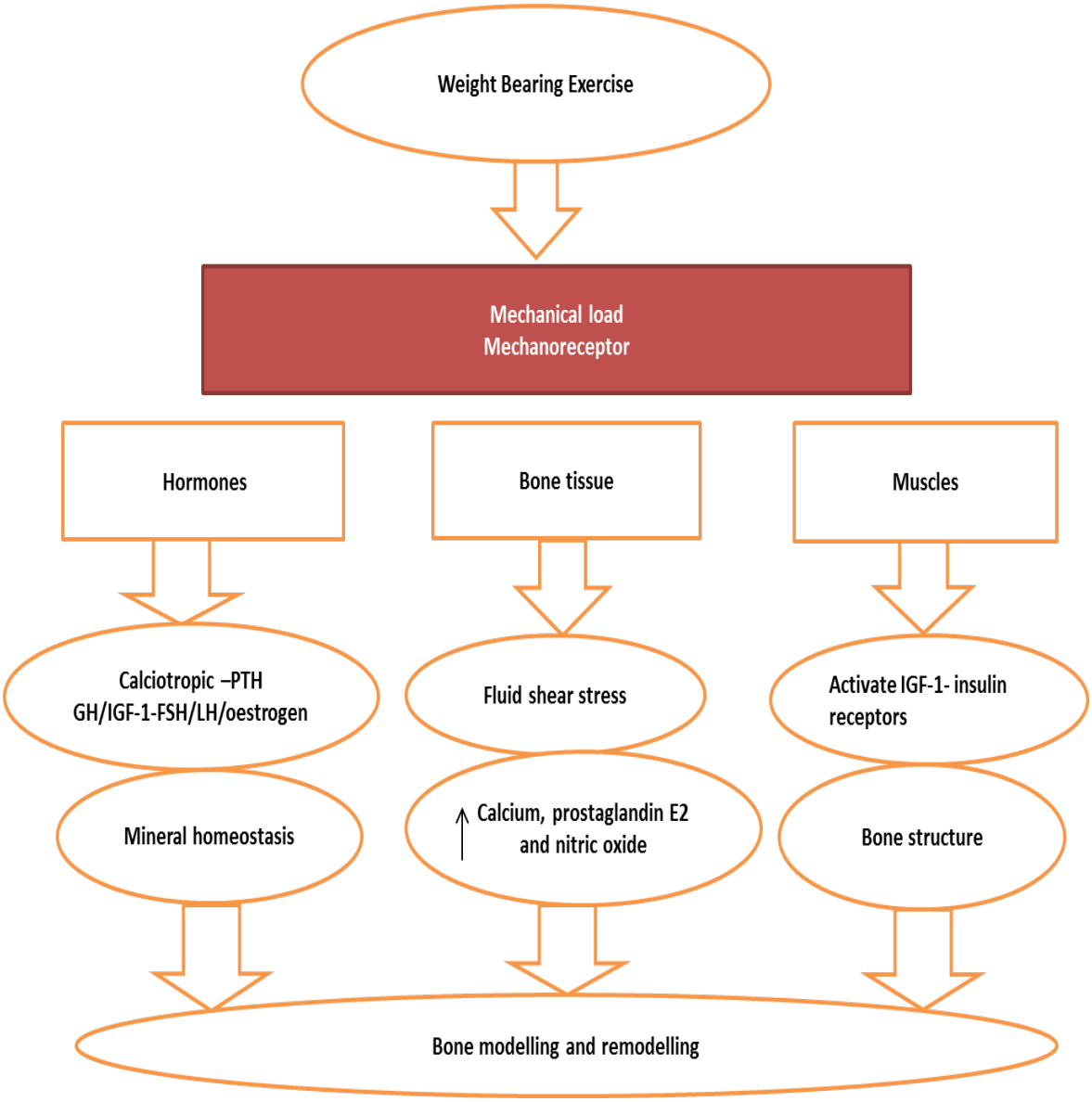


Figure 1-9 Mechanism of influence of weight-bearing exercise on bone

1.4.2 Effectiveness of weight-bearing exercise on the bone health of children and adolescents

1.4.2.1 Short terms benefits of WBE on bone

The majority of randomized control trials (RCT) and longitudinal studies were carried out on a variety of animal and human cohorts to assess the benefits of WBE on bone density and bone strength.

1.4.2.1.1 Animal studies

Animal model studies have been fundamental to the design of exercise regimens that aim to enhance human bone health. The majority of these studies indicate that high impact and weight bearing exercises provide an increase in bone mass and strength, as summarised in Table 1-11. Among WBEs, jumping programs seem to be the most beneficial in terms of bone density and strength (300,301). Studies have demonstrated that the mechanical load of jumping exercises in rats showed significant higher bone formation (302,303), volumetric bone mineral density and mechanical structure and bone strength (304). Ju and colleagues found significant increases in the total cortical area of the tibia and femur, which was reflected in increased periosteal circumference following as few as 5-20 jumps per day in young male rats (305,306). Similarly, another research group revealed that as few as five jumps per day in growing rats improved bone mass and strength with few differences between animals that jumped between 10 and 40 times per day (307). Jump training with minimal loading showed more favourable bone geometry, and was more effective at augmenting cortical bone integrity compared with high load jump training in skeletally mature rats (308). It was also reported that the beneficial effects of jumping exercises could be maintained for a period of 24 weeks when followed up with exercise consisting of 11% to 18% of the initial exercise load (309).

Table 1-11 Summary of animal studies of jumping exercises and bone

	Total	Age	Sex	Exercise programs	Duration (wk)	Groups and numbers	measurement	Results
(305)	28	5 wk	M	Treadmill running: 25 m/min, 1 h/day, 5 days/wk. Jumping: 10 jumps/day, 5 days/wk	5	n=7 tail suspended n=7 jumping n=7 treadmill running n=7 control	Femur BMD - Trabecular architecture	Femur BMD was significantly increased in both running and jumping groups compared with controls. Jumping exercise increased BV/TV (38%) and Tb.Th (22%) and decreased Tb.Sp (16%) and TBPf (38%) compared with controls.
(306)	24	8 wk	M	30 jumps/day, five days/wk	3	n=8 tail suspended n=8 sedentary control n=8 jump exercise	Femur BMD - Trabecular architecture	Jump exercise during the tail suspension period increased trabecular thickness (14%, $p < 0.001$) and suspended reduction of trabecular number.
(310)	144	12 wk	F	40 jumps per day, 5 days/wk for 8 wk then either maintaining or decreasing the frequency or intensity	8-24	n=10 8wk vs. n=10 sedentary, n=10 sedentary 32 wks. nine groups n=10 each for 8 wks of standard training (STP) followed by 24 wks of continuous exercise (CTP)	Bone turnover markers and tibia bone mass	Increases in tibia mass were observed in rats that continued to exercise at workloads of 30 jumps/wk and above after 8 STP. Serum alkaline phosphatase concentrations increased whereas serum CTX concentrations decreased in rats given workloads of 40 jumps/wk and above.
(311)	80	Young adults	F	1 - Swimming: 5 days/wk for 60 min/day 2 - Jumping: 20 jumps per session, 5 days/wk for 3 wks 3 - Vibrating: a longitudinal amplitude of 1 mm and frequency of 50 Hz 5 days/wk, 20 min/session	3	n=10 hind limb suspension n=10 controls n=10 swimming n=10 swimming controls n=10 swimming+jumping n=10 jumping n=10 jumping controls n=10 vibration therapy	Femur BMD, bone strength, and bone markers	There was no significant difference between the three physical exercises, but the oestrogenic effect of vibration was slightly lower than that of swimming and jumping.
(312)	42	6 Mon	M	15 sessions of resistance jumping with a starting weight of 80. 50 repetitions on session 1 and increasing up to 410 in session 15.	5	n=16 high-load jump n=15 low-load jump n=11 sedentary control	Tibia and femoral neck	Greater bone formation in jumping groups vs. controls. Greater bone volume vs. trabecular volume (BV/TV) in jumping groups vs. control.
(313)	48/ Wk. 30/Daily	10 wk.	F	Jumping session consisting of jumping 45 cm high	8	n=8 sedentary group n=10 one jump/wk. n=10 three jump/wk. n=10 five jump/wk. n=10 seven jump/wk.	Fracture test and tibia cross sectional	The cortical area, periosteal perimeter and moment of inertia were significantly greater in all exercise groups than their respective sedentary groups. There was little additional benefit of bones being loaded by two separate exercise sessions daily.

Wk: week; Mon: month; F: females; M: males; Tb.Th: Trabecular thickness; Tb.Sp: Trabecular separation; TBPf: Trabecular bone pattern factor

1.4.2.1.2 Human studies

Following promising findings of animal studies, several human studies, varying in size, duration, exercise intervention, frequency and the subjects' age group, tested WBE as a causal factor for bone strength (Table 1-12).

The osteogenic effects of WBE, particular jumping exercise interventions, was well summarized by Hind (286) who reviewed 22 studies indicating that after at least seven months of jumping intervention, jumpers had an increase of 1.4 - 6.2% BMD: 0.9 - 5% BMC at femoral neck (FN), and an increase of 0.9 - 5.5% BMD: 0.9 - 3.3% BMC at lumbar spine (LS) compared with control children. On the other hand, other studies have reported little or no effect on bone density (314-316). A recently meta-analysis of WBE concluded that WBE increases bone mass and BMD during the prepubertal years, but has little or no effect during puberty (281). Another group of researchers reported that a short term (seven months) of jumping exercises in pre-pubertal individuals (males and females) had a great effect on bone density with a 3.5% increase at LS and a 4.5% increase at FN in the interventional group compared with the control (284), and the changes at FN of the intervention group were retained and maintained for one year (317) or 4 years after detraining (318). In another study, the skeletal gain of jumping exercises in the intervention group at total body, LS and FN after eight months of regular exercise (319) were sustained for the following three years after intervention (320).

In contrast to findings in DXA-based studies, few studies using pQCT to evaluate the effects of high-impact exercise on bone structure and geometry revealed either improvement (321-324)or no significant effects (325). The geometric bone changes (structural/properties) in early pubertal girls doing jumping exercise for seven months improve significantly compared with the controls (326). Several studies indicated that both frequency and amount of loading in WBE during growth could be important contributors to increased bone mass. It was reported that the optimal dose of impact loading required for bone FN-BMD in children includes simple jumping programs (100 two-footed jumps off 61-cm boxes, three times per day for seven months (284); or 10 counter-movement jumps, three times a day for eight months (327).

However, many of these studies had a high risk for bias and poor exercise compliance was a common concern. In addition, a problem for the interpretation of many of these studies is the multi-modal nature of interventions; incorporating activities within the same programme making it impossible to isolate the effectiveness of individual exercise modalities although jumping exercises are a common component of the most successful interventions.

Table 1-12 Summary of RCT involving jumping exercises on bone.

Study	No of subjects/groups	Age range (years)	Intervention	Measurements	Results
(284)	Intervention n=25 males n=20 females Control n=26 males n=18 females	5.9-9.8	100 two-foot jumps off 61" box 3 days/wk for 7 months	DXA-LS-FN-BMC/BMD/BA	4.5%-3.1% change in LS-FN, higher BMC in intervention than control.
(328)	All female Intervention: n=32 Control: n=43	8.8–11.7	Jumping for 10 minutes, 3 times/wk for 20 months	DXA TB-LS-FN-BMC	There were substantially greater gains in LS-BMC (41.7% vs. 38.0%) and FN-BMC (24.8% vs. 20.2%) in intervention than in control girls ($p < .05$).
(327)	Intervention n=23 males n=28 females Control n=23 males n=28 females	8.9-10.8	10 counter-movement jumps 3 times/day for 8 months	DXA LS-FN BMC/BA	1.4% increase in LS-BMC2% increase in FN-BMC in intervention vs. control.
(326)	Prepubertal females Intervention: n=43 Control: n= 63 Early puberty females Intervention: n =43 Control: n=25	9-12	Jumping for 10 minutes, 3 times/wk for 7 months	FN-geometry and density	No changes in bone structure in the prepubertal girls. Early puberty intervention group showed significantly greater gains in FN (2.6%, $p = 0.03$) and IT (1.7%, $p = 0.02$).
(325)	Intervention n=12 males n=14 females Control n=11 males n=17 females	3-18	25 jumps/day from 45cm box, 5days/wk for 12 weeks	DXA TB-BMC pQCT Tibia	Greater increases in TB- BMC than control at all pubertal stages. No significant change in tibia.

(329)	Intervention and control: n=21-sets of twin females	8-9.4	WBE involving rope skipping 50 times, hopping 20 times, jumping off box 30 times. 10 min session 3 times/wk for 9 months	DXA TB-LS-FN BMD/BMC	No difference in bone parameters.
(322)	Intervention: n=76 females Control n=75 females	10-11.2	10 mins jumping 3 times/wk plus capoeira (Brazilian sport) for 9 months	DXA TB-LS-FN BMC pQCT tibia and radius	Radius -BMC at the 4% site tended to increase more in EX than CON (+ 36.1% vs. + 10.7%, $p = 0.065$), and there was a tendency for greater improvements in radial cortical density at the 66% site in EX than CON (+ 2.7% vs. + 0.3%, $p = 0.072$).
(330)	Intervention n=12 males n=10 females Control n=11 males n=12 females	8-12	10 mins jumping activity twice /wk for 8 months	pQCT tibia strength and geometry	No significant differences.
(319), (320)	Intervention n=22 males n=23 females Control n=24 males n=30 females n=30 control	13.4-14.2	300 jumps /10 min twice/wk for 8 months	DXA LS/FN-BMC	Intervention group gained significantly more FN-BMC than controls (185.4 ± 91.9 versus 110.4 ±96.1 g; $p = 0.009$) with no changes in other significant parameters. These changes were maintained for one, and following three years.

DXA, dual-energy X-ray absorptiometry; pQCT, peripheral quantitative computer tomography; BMD, bone mineral density; BMAD, bone mineral apparent density ; BMC, bone mineral content; LS, lumbar spine; TB, total body; FN, femoral neck; BA, bone area

1.4.3 Long term benefits of WBE on osteoporosis risk

Although there is overwhelming evidence that exercise can optimize skeletal development, it remains incompletely understood whether exercise-induced skeletal gains during childhood are maintained into later life. Researchers suggest the amount of bone mineral acquired during childhood and adolescence accounts for approximately 60% of the risk for osteoporosis in later life (331) and the susceptibility to osteoporosis may be detectable in early childhood as bone status during childhood is a strong predictor of bone status in young adulthood, when peak bone mass is achieved (332). It has been calculated that a 10% increase in peak bone mass would delay the onset of osteoporosis by 13 years (220) and could reduce fracture risk by as much as 50% (333). However, few studies have addressed the question of whether reported short-term benefits of WBE continue to accrue with participation over a longer term. Prospective observational animals and human studies suggest some of the benefits of exercise on bone during childhood may be maintained into young adulthood (334,335), a possible preventive strategy against fragility fractures in old age (333) . On the other hand, the reversibility effect of exercises on bone has been reported elsewhere, showing a marked decline in BMD upon discontinuation of WBE (336). Perhaps there is no reason to believe that some regression would not occur in adolescence (337). Nevertheless, some other benefits (on geometry, microarchitecture, and/or strength) may persist despite loss of bone mass (335,338).

1.4.4 Other benefits of exercise

Exercise regimens targeting bone are unique and different from other regimens proposed for other systems such as metabolic and cardiovascular. However, some exercises regimens are often combined to target a wider range of tissues, with the aim of improving several aspects of health outcomes simultaneously.

1.4.4.1 Body composition

The effects of WBE on body composition have shown conflicting results. Numerous studies of children and adolescents show that WBE induces increases in muscle mass and strength (339), and increasing duration or increasing frequency of short-duration regimens may contribute to greater benefits in bone and body composition (340,341). A study showed that a twice-weekly, school-based, 10-min jumping regime resulted in increased lean mass in a group of boys and girls compared with matched controls, and boys gained more lean mass and had a significant decrease in fat mass compared with controls (342). On the other hand, it has been reported that exercise in early pubertal children shows little or no effect on body composition (343) and jumping-focused interventions may reduce fat and enhance musculoskeletal tissue in school-age children but does not increase lean mass (329,341).

1.4.4.2 Lipids profiles

Several studies have established the beneficial of exercise in improving plasma lipid profiles by increasing the ratio of HDL to total cholesterol and reducing the ratio of LDL to total cholesterol (142,344). Low and high-intensity exercises may be particularly beneficial in reducing the risks of hyperlipidemia and cardiovascular disease (345,346), which was shown to be independent of weight loss (347). In contrast, studies have reported that resistance and high impact exercises have limited efficacy in improving lipid profiles (348).

1.4.4.3 Insulin sensitivity

There is an accumulation of evidence to support the belief that resistance exercise is effective in decreasing fasting insulin and glucose and improving insulin resistance in children, adolescents (349) and young adults (350) and that could be applied to the prevention of T2DM (351). A recent meta-analysis of effectiveness of exercise training on fasting insulin and insulin resistance in children and adolescents revealed a 11.4-U/mL (95% CI: 5.2–17.5) improvement in fasting insulin and an improvement in HOMA-IR of 2.0 (95% CI: 0.4–3.6) (349). Studies exploring the impact of mechanical load exercise on insulin resistance are few. There appears to be improvement in insulin sensitivity with aerobic exercise regimens and combinations of aerobic and resistance training in obese (352) and non-obese children (353).

1.4.4.4 Quality of life

Both weight-bearing and non-weight-bearing exercise programs are reported to improve health-related QoL (354,355). A review of adult data obtained from cross-sectional studies showed a consistently positive association between physical activity level and health-related quality of life (356).

1.4.5 Summary

Although several favourable benefits of WBE, in particular jumping exercise, for bone are well established, it is still not known the extent to which it is recommended for the prevention of osteoporosis. Long-term follow-up is necessary to determine whether WBE during childhood and adolescence can optimize peak bone strength through growth to affect osteoporosis risk in later life.

CHAPTER 2

Aims

2.1 Rationale, Specific Aims

It is well known that growth hormone (GH) brings about several effects, involving bone, body composition, lipid and glucose homeostasis as well as health related quality of life. However, the complex interplay between these parameters is rather poorly studied in children and adolescents with childhood-onset-GH deficiency (CO-GHD). Data showed that CO-GHD may contribute to low bone density and osteoporosis in adulthood. However, the direct mechanisms of which CO-GHD effects on bone health remain largely unknown. Therefore, the overall aim of this PhD thesis is to achieve more knowledge about the impacts of CO-GHD on bone health at time of initial evaluation and retesting at final height. In addition through this thesis the following specific aims are proposed:

2.1.1 An audit of the management of CO-GHD during young adulthood in Scotland (Chapter 3).

Hypothesis: Patients with CO-GHD require biochemical re-evaluation and reconfirmation of GHD during transition before reinstituting adult rhGH therapy.
Aim: To review the management of CO-GHD after final height in Scotland; in addition to assess the incidence of, and to find out the predictors of, persistent GHD, in patients with CO-GHD after retesting at final height.

2.1.2 Bone mass and body composition in adolescent with CO-GHD at final height (Chapter 4).

Hypothesis: Bone health is adversely affected in patients with CO-GHD during transition after attenuation final height.
Aim: To compare size/height corrected DXA parameters of bone mass and body composition in CO-GHD adolescents with healthy controls.

2.1.3 Bone health and body composition in children and adolescents with CO-GHD at time of initial evaluation and retesting (Chapter 5).

Hypothesis: Bone health is adversely affected in patients with CO-GHD at time of initial evaluation and retesting at final height.
Aims: 1-To evaluate musculoskeletal health in children and adolescents with CO-GHD at time of initial evaluation and retesting at final height. 2 -To explore the relationship of bone and body composition parameters with bone metabolism and turnover biomarkers in subjects with CO-GHD.

2.1.4 Metabolic parameters and glucose homeostasis in children and adolescents with CO-GHD at time of initial evaluation and retesting (Chapter 6).

Hypotheses: 1-Deterioration of metabolic parameters and glucose homeostasis in children and adolescents with CO-GHD.

2- Metabolic and adiposity markers have determined effects on bone health of subjects CO-GHD.

Aim: To investigate lipids, adipokines (leptin- adiponectin- resistin) and glucose homeostasis and their relationship with bone and body composition in children and adolescents with CO-GHD at time of initial evaluation and retesting at final height.

2.1.5 Quality of Life of Children and Adolescents with CO-GHD (Chapter 7).

Hypothesis: Dimension in health related quality of life in CO-GHD in relation to GH statue.

Aim: To evaluate quality-of-life in children and adolescents with CO-GHD at the time of initial evaluation or retesting at final height.

2.1.6 The effect of weight bearing exercise in children and adolescents with CO-GHD (Chapter 8).

Hypotheses: 1-Exercise mitigates the effect on bone health in CO-GHD patients.

2- The beneficial effect on bone health is greater in those who have exercise and rhGH.

Aim: To explore the feasibility performing weight bearing exercise (jumping exercise) in children and adolescents with CO-GHD, and to assess its effects on the bone health and body composition with or without rhGH therapy.

CHAPTER 3

An Audit of the Management of Childhood-Onset Growth Hormone Deficiency during Young Adulthood in Scotland

3.1 Abstract

Background: Adolescents with childhood onset growth hormone deficiency (CO-GHD) require re-evaluation of their growth hormone (GH) axis on attainment of final height to determine eligibility for adult GH therapy (rhGH).

Aim: Retrospective multicentre review of management of young adults with CO-GHD in four paediatric centres in Scotland during transition.

Patients: Medical records of 130 eligible CO-GHD adolescents (78 males), who attained final height between 2005-2013 were reviewed. Median (range) age at initial diagnosis of CO-GHD was 10.7yrs (0.1-16.4) with a stimulated GH peak of 2.3μg/l (0.1- 6.5). Median age at initiation of rhGH was 10.8yrs (0.4-17.0).

Results: Of the 130 CO-GHD adolescents, 74/130 (57%) had GH axis re-evaluation by stimulation tests /IGF-1 measurements. Of those, 61/74(82%) remained GHD with 51/74(69%) restarting adult rhGH. Predictors of persistent GHD included an organic hypothalamic-pituitary disorder and multiple pituitary hormone deficiencies (MPHD). Of the remaining 56 /130 (43%) patients who were not re-tested, 34/56 (61%) were transferred to adult services on rhGH without biochemical retesting and 32/34 of these had MPHD. The proportion of adults who were offered rhGH without biochemical re-testing in the four centres ranged between 10% and 50% of their total cohort.

Conclusions: A substantial proportion of adults with CO-GHD remain GHD, particularly those with MPHD and most opt for treatment with rhGH. Despite clinical guidelines, there is significant variation in the management of CO-GHD in young adulthood across Scotland.

3.2 Introduction

The transition of care from childhood to adulthood for many chronic disorders requires a careful coordinated approach and this is particularly important in growth hormone deficiency (GHD). Traditionally, children with childhood onset GHD (CO-GHD) discontinue recombinant human GH therapy (rhGH) after attaining final height. However, adults with CO-GHD may have increased fat mass, decreased muscle mass and low bone mineral density, as well as reduced cardiac performance, altered lipid status, reduced physical performance, impaired cognitive function and reduced well-being (133,203). Reports suggest that these adults may benefit from rhGH (357,358).

A number of studies have shown that a high proportion of CO-GHD patients remain GH deficient as adults especially those with multiple pituitary hormone deficiencies (MPHD) and/or structural abnormalities, whereas the majority of those with idiopathic or isolated GHD no longer have GHD in adulthood (359-361). Therefore, after childhood treatment it is necessary to review GH status in order to assess appropriateness of adult rhGH replacement (264). However, the extent of benefit from this therapy may be variable and the decision to reinstitute rhGH needs to be undertaken carefully.

In this context, clinical practice guidelines have been issued on the subject of transition of care of young adults with CO-GHD (200-202,362). However, the practicalities of these guidelines as well as the extent to which these guidelines have been implemented in clinical practice are unclear. The purpose of this multicentre study was to understand the variation that may exist in the management of young adults with CO-GHD after attainment of final height.

3.3 Patients and Methods

We reviewed databases from the four specialist endocrine centres in Scotland and identified young adults who had been diagnosed as having CO-GHD and who had been treated with GH during childhood and had subsequently reached final height between 2005-2013. Study entry criteria were: CO-GHD (low GH peak response on stimulation test <6.6µg/l), GH treatment during childhood, attainment of final height between 2005-2013 (height velocity <1cm/year as defined in all centres), and evaluation of GH- axis by stimulation tests and/or IGF-1 levels after withdrawal of GH for at least one month. Exclusion criteria included: untreated CO-GHD, GH-treated patients with CO-GHD who have not yet attained final height. Baseline demographic data included: aetiology of CO-GHD, age at diagnosis of CO-GHD, duration of GH treatment, presence of multiple pituitary hormone deficiencies (MPHD), re-evaluation of GH axis, and whether adult GH treatment was recommenced or not (Appendix A). The persistent GHD after retesting for four centers was defined as cutoff <5 µg/L GH peak response for dynamic stimulation testes and/or low serum IGF-1 levels (<2 SD for age and sex)(200). IGF-1 level measurement for centres A, B, and D were done using IDS iSys and centre C measured IGF-1 levels by immunoassay on the Siemens Immulite. All IGF-1 levels were corrected for age and sex accordingly.

3.4 Statistical Analysis

Data were analyzed using Minitab software (Version 16) with a significance level of <0.05 and are described as median, ranges and percentage. Additionally, the Mann–Whitney U–test was used for calculation of significance of differences between median values. Association with clinical factors was assessed by Spearman's rank coefficient and a positive predictive value (PPV) was calculated for the identified predictors of persistent GHD.

3.5 Results

3.5.1 General characteristics

A total of 142 patients were screened, 130 of whom met inclusion criteria. The 130 patients (78 male) comprised of: 70 from centre A, 32 from B, 18 from C and 10 from D. Table 3-1 displays the aetiology of CO-GHD. An approximately of 29% of our cohort had congenital GHD of organic aetiology and around 40% had acquired GHD of oncology and cranial irradiation. In addition, 20% of our cohort, their GHD was a one feature of either a syndrome or multiple organs defects. Median age at diagnosis of CO-GHD was 10.7 years (0.1 - 16.4) with an initial stimulated GH peak of 2.3µg/l (0.1 - 6.5), and basal IGF-1 was 74µg/l (4.0 - 410.0). Median age at initiation of rhGH was 10.8 years (0.4- 17.0). GH peak at diagnosis was lower in those with MPHD compared to IGHD (1.9µg/l (<0.1- 6.4) vs. 3.0µg/l (0.3- 6.5) respectively: $p<0.01$).

Table 3-1 The categories of patients with CO-GHD according to aetiology and centres distribution is shown as (A, B, C, D).

	Total number of cases 130	IGHD 48/130(37%)	MPHD* 82/130 (63%)
Congenital n (%) (A,B,C,D)	**38/130 (29%)**	**12 (8,1,2,1)**	**26 (14,3,4,5)**
-Pituitary axial structural abnormalities (A,B,C,D)	24	9(6,1,1,1)	15 (6,3,1,5)
-Midline axial structure defects (SOD)(A,B,C,D)	14	3(2,0,1,0)	11(8,0,3,0)
Oncology/cranial irradiation n(%)(A,B,C,D)	**51/130 (40%)**	**8 (5,3,0,0)**	**43(18,19,4,2)**
- Craniopharyngioma (A,B,C,D)	15	-	15 (6,7,1,1)
- Hematologic malignancies (A,B,C,D)	12	4 (4,0,0,0)	8 (6,0,1,1)
- Medulloblastoma (A,B,C,D)	6	1 (0,1,0,0)	5 (1,4,0,0)
- Other CNS tumors (A,B,C,D)	18	3 (1,2,0,0)	15 (5,8,2,0)
Idiopathic[1] n (%) (A,B,C,D)	**15/130 (11%)**	**13 (7,1,5,0)**	**2 (1,1,0,0)**
Others[2] n (%) (A,B,C,D)	**26/130 (20%)**	**15 (12,1,1,1)**	**11 (5,3,2,1)**
-Crohn's disease (A,B,C,D)	4	4 (3,0,0,1)	-
-Coeliac disease (A,B,C,D)	2	-	2 (0,1,1,0)
-Haematological diseases [3](A,B,C,D)	2	1 (1,0,0,0)	1 (1,0,0,0)
-other diseases [4] (A,B,C,D)	11	8 (6,1,1,0)	3 (0,2,1,0)
-Syndromes [5] (A,B,C,D)	6	2 (2,0,0,0)	4 (3,0,0,1)
-Acquired Brain injury (A,B,C,D)	1	-	1 (1,0,0,0)

Data are presented as the numbers of patients and percentages are given in parentheses
*33/82 patients with one additional pituitary hormone deficiency, 17/82 with two additional deficiencies, 19/82 with three and 13/82 with four additional deficiencies 'panhypopituitarism'.
IGHD, isolated growth hormone deficiency; MPHD, multiple-pituitary hormone deficiencies; SOD, Septo-optic dysplasia
1 Normal pituitary MRI, GHD is not associated with other conditions; 2 Normal pituitary MRI (or no MRI report), but GHD is associated with other conditions; 3 (Thalassemia, X-linked Sideroblastic Anaemia); 4 (Microephaly with learning disability, history of intrauterine growth retardation, gastrochisis with history of small for gestational age, Asthma, joint hypermobility syndrome, pesudohypoparathyrodism); 5 (Charge syndrome, Noonan syndrome, Kallman Syndrome, trisomy 22, Klinefelter's syndrome, Turner's syndrome with GHD).

3.5.2 Re-evaluation of GH axis

A total of 74/130 (57%) patients with CO-GHD (IGHD=31 (42%): MPHD=43(58%)) were biochemically retested at a median age of 18.2 years (14.5- 21.3) (with one outlier patient who was retested at the age of 27.5 years), rhGH treatment was discontinued at the median age of 16.4 years (10.8 - 21.0). Biochemical retesting was performed after a median period of 0.5 years (0.1- 5.6) off rhGH (21/74 (28%) were retested over period of (0.1-0.3 years) and 34/74(46%) over a period of (0.4-5.7 years), with incomplete data on timing of re-testing in 20/74 (27%). Median duration of childhood treatment was 5.3 years (0.4- 16.8). At retesting, the median GH peak was 1.6µg/l (0.1-23.7) and IGF-1 was 88.0µg/l (15.0- 631.0). Of those retested, 61/74 (82%) (32 males) remained GHD and were eligible for adult rhGH, with 51/61 (84%) re-starting adult rhGH and 10/61 (16%) declining therapy although it is possible that they may have restarted at a later stage. The remaining 13/74 (18%)(10 males) who were no longer GH deficient consisted of eight with idiopathic IGHD, two brothers with central hypothyroidism and normal pituitary MRI, one with an ectopic pituitary, one with hypogonadism and Coeliac disease and one with a history of cranial irradiation. Of the 56 of 130 (43%) cases of CO-GHD who were not retested 34 (61%) were transferred from paediatric to adult services without biochemical retesting during transition, 12 (21%) stopped treatment without biochemical re-evaluation and 10 (18%) were lost to follow up whilst on treatment (Figure. 3-1).

Dynamic function stimulation tests were performed in 40/74 (54%) patients who were retested, with 35/40 (88%) of subjects having a low GH peak response <5µg/l, with 27/35 of them having severe GHD with a GH peak response <3µg/l. Of the remaining 34/74 (46%) patients who were retested, IGF-1 levels alone were available and low enough to confirm GHD (< -2 SD for age and gender) in 19/34 (56%) of which 15 had MPHD and 4 had IGHD (organic causes and abnormal pituitary MRI). Two patients (MPHD) had IGF-1 levels within normal range on initial retesting (> 2 SD for age and gender), but were confirmed to have GHD following GH stimulation tests.

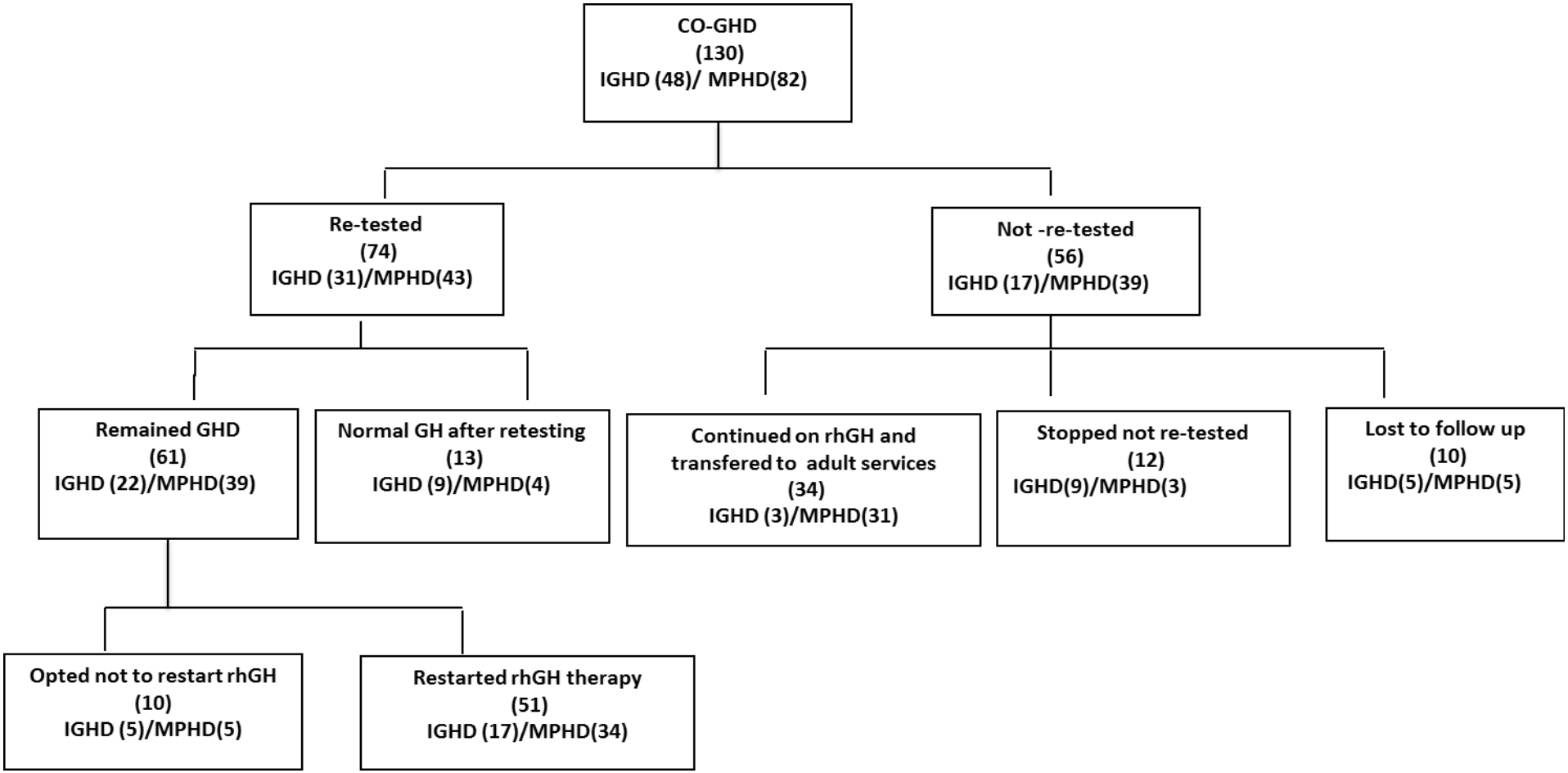


Figure 3-1 Flow chart of study cohort and the outcome of management of CO-GHD in Scotland

3.5.3 Reconfirmation of GHD and initiation of adult GH replacement therapy

Of the 130 with CO-GHD, 34 (26%) patients continued adult rhGH without temporary cessation of therapy or formal retesting. Of these 34, nine had structural abnormalities on MRI, 22 were related to late effects of cancer therapy and three had unexplained GHD. Of those 34, 31 (91%) had MPHD (17/32 of them had three or more additional pituitary hormone deficiencies (PHDs)); and 3/34 (9%) had IGHD (two with pituitary structural abnormalities on MRI and one with tumour related GHD). These patients were advised to continue rhGH until their mid-20s.
For patients who were re-tested, GH cut offs for considering rhGH varied between centres. Not all patients found to have persistent GHD restarted adult GH therapy despite low peak GH levels at re-testing. There were four patients who were found not to have severe GHD with GH peaks 4-5 µg/l (three patients from centre B, one from centre A) and were not offered rhGH as they did not meet adult criteria for replacement. However, among those who were offered rhGH after retesting, one patient with IGHD (centre C) had a GH peak >5 µg/l (5.5 µg/l).

3.5.4 Variation in the management between centres

There were substantial variations in the management of CO-GHD between Scottish centres. Re-testing with stimulation testes and/or IGF-1 levels was found to be the highest in centre A (68%), while centre C had the lowest percentage of retested patients (28%), although this did include all IGHD patients from centre C. Centre B did not retest those with a high likelihood of permanent GHD (especially those with three or more additional PHDs) and had the highest percentage of adults on rhGH without biochemical re-evaluation (Table 3-2). A total of 32/130 patients in the cohort had three or more additional PHDs (Table 3-3). Of these 32, 14 (44%) were retested using their IGF-1 levels alone and all were confirmed to have adult GHD, 17 (53%) continued on rhGH without biochemical retesting and one was lost to follow up whilst on treatment.

Table 3-2 Management of patients with CO-GHD according to each Scottish centre

	All centres	A	B	C	D
Total number of patients (n)	**130**	**70**	**32**	**18**	**10**
Total number of patients re-tested n (%)	**74/130 (57)**	**48/70 (69)**	**16/32 (50)**	**5/18 (28)**	**5/10 (50)**
Persistent GHD n (%)	61/74 (82)	43/48 (90)	12/16 (75)	1/5 (20)	5/5 (100)
Those with persistent GHD who restarted rhGH n (%)	51/61 (83)	35/43 (81)	11/12 (92)	1/1 (100)	4/5 (80)
Total number of patients <u>not</u>-retested n (%)	**56/130 (43)**	**22/70 (31)**	**16/32 (50)**	**13/18 (72)**	**5/10 (50)**
Continued adult rhGH therapy without re-testing n (%)	34/56 (61)	7/22 (32)	16/16 (100)	7/13 (54)	4/5 (80)
Lost to follow up whilst on treatment n (%)	10/56 (18)	9/22 (41)	0	0	1/5 (20)
Stopped GH, no re-testing required n (%)	12/56 (21)	6/22 (27)	0	6/13 (46)	0

Data are presented as the numbers of patients and percentages are given in *parentheses*

Table 3-3 Variation in the management of patients with CO-GHD between the four Scottish centres according to GHD categories

Centres	**A**		**B**		**C**		**D**	
	IGHD	**MPHD**	**IGHD**	**MPHD**	**IGHD**	**MPHD**	**IGHD**	**MPHD**
Total CO-GHD n=130 [32]	**32**	**38[16]**	**6**	**26[10]**	**8**	**10[3]**	**2**	**8[3]**
Retested n=74 [14]	**21**	**27[13]**	**4**	**12[0]**	**4**	**1 [0]**	**2**	**3[1]**
With structural abnormalities[1]	8	13[5]	-	2	1	-	-	-
Tumour related[2]	3	13[8]	2	7	-	1	-	1
Idiopathic/unexplained[3]	10	1	2	3	3	-	2	2[1]
Re-confirmed GHD	**16**	**27[13]**	**3**	**9[0]**	**1**	**0**	**2**	**3[1]**
Not retested (<u>but on adult rhGH</u>) n=34 [17]	**0**	**7[2]**	**2**	**14[10]**	**1**	**6 [3]**	**-**	**4[2]**
With structural abnormalities[1]	-	-	2	1	1	3 [3]	-	1
Tumour related[2]	-	5[2]	-	12[10]	-	3	-	1[1]
Idiopathic/unexplained[3]	-	2	-	1	-	-	-	2[1]

Data are presented as number of patients with CO-GHD and [number of patients who have three and more additional pituitary hormones deficiencies].

1 MRI imaging reported hypothalamic-pituitary axial structural abnormalities ; 2 Cranial irradiation; 3 Normal pituitary MRI /Congenital GHD unexplained (no MRI report)/ and /or associated with chronic disease

3.5.5 Predictors of persistent GHD on re-evaluation

Patients with persistent GHD were diagnosed at an earlier age ((8.4 years (0.3- 16.0) vs. 11.6 years (7.1- 15.5), p=0.01) and reached final height with re-evaluation of their GH axis in earlier adolescence ((17.9 years (14.2- 21.2) vs. 19.3 years (17.3- 21.3), p=0.004) than those who were no longer GH deficient on retesting. No significant differences in the other parameters between persistent GHD and non-persistent GHD were identified at time of diagnosis or re-evaluation. In this population the peak GH level on retesting was positively related with the GH peak level at childhood (r = 0.4, P = 0.02).

The number of additional PHDs was a predictor of a low peak GH on retesting as all patients with two or more additional PHDs had a lower GH response (<5µg/l) at reassessment with a PPV (93%). The presence of hypothalamic–pituitary structural abnormalities has a high PPV (96%) of persistent GHD, as of the 25 who were retested, 24 were reconfirmed with persistent GHD. Similarly, CO-GHD with a history of cranial irradiation predicted persistent GHD in adulthood (96%).

3.6 Discussion

In this study, we reviewed the management of CO-GHD during transition in Scotland. The aetiology and the categories in the distribution of our cohort among the Scottish centres are consistent with previously reported by KIMS database of adult with CO-GHD (Pfizer International Metabolic Database)(234), and with National Cooperative Growth Study (NCGS) (USA-Canada) (363). We cannot comment on racial differences, as the data was not available. However, the (NCGS) revealed that 85% of patients receiving GH therapy for idiopathic GHD were white Caucasian, 6% were black African, and 2% were Asian (363).

Our data confirm that a high proportion (82%) of the retested patients with CO-GHD continue to have GHD as adults. The majority (80%) of those who remain GH deficient opted to resume adult GH treatment, however it is unknown whether they complied with therapy and for how long they continued with the treatment. It may also be the case that those adults who were GHD initially declined to restart rhGH during transition, but later reconsidered GH therapy. Factors influencing this decision would be an important area for future studies.

Previous published studies have reported variable estimates of persistent GHD in adulthood ranging from 12.5-90% (364,365) but the high incidence of ongoing GHD in adulthood in our cohort may be attributed to the large proportion of patients with organic causes for their GHD. Some of our subjects with MPHD who had no structural abnormalities on MRI continued to have GHD, raising the possibility of an underlying genetic disorder. Similarly, the majority of idiopathic IGHD who were re-evaluated were GH deficient which may also indicate an underlying genetic predisposition. These findings suggest the importance of follow up and regular assessment of pituitary function in those with a low probability of persistent GHD, as they may develop other

pituitary hormone deficiencies as previously demonstrated(366,367). On the other hand, some patients who would be considered as having a moderate to high probability of persistent GHD (IGHD with structural abnormalities, patients with MPHD or those with a history of cranial irradiation) were no longer GH deficient. These findings demonstrate the limitations in using "at risk" groups to determine who require re-evaluation of their GH axis and those who do not.

Our data confirm that while there are no unequivocal auxological or clinical signs that predict the transiency or the persistence of GHD, a history of organic disease, the presence of two or more additional PHDs (368,369), presence of hypothalamic-pituitary structural abnormalities and tumour related organic GHD are strong indicators of persistence GHD(199,206,370,371).

In terms of timing of retesting, the current guidelines suggest that a period from one to three months off rhGH is sufficient for retesting (200). Our data show a variable interval between stopping treatment and reassessment, with only 28% of patients off rhGH for less than 3 months. It is not clear for those who were off rhGH for longer duration before reassessment whether their stopping rhGH was for reasons other than re-testing. However, this prolonged period off rhGH may be associated with detrimental effects on somatic bone and body composition development during transition (234,245), with recommendations for prompt resumption of rhGH in individuals with clinical evidence of persistent GHD (217). Furthermore, a longer interval off rhGH may increase the risk of being lost to follow up in these already vulnerable patients and continued follow up around this time is essential (372). We recommend that in patients who are under the care of paediatric services, the evaluation of GHD in transition should be undertaken by the paediatric clinic, ideally in the context of a joint transition service to improve the follow up and smooth transfer of adolescents with chronic endocrine conditions to the adult services as previously suggested (373).

The principle of offering rhGH during transition for those who have ongoing severe deficiency was variable between centres as the cut-offs chosen are variable, though the majority were in keeping with the guidance suggesting a GH peak <5 μg/l constituting severe GHD in transition (200,201). Few patients in our cohort declined restarting adult rhGH, they may be asymptomatic and therefore reluctant to restart rhGH therapy. Approximately one third of our subjects were considered to be very likely to have permanent GHD and therefore continued rhGH uninterrupted, apart from adjustment to an adult GH dose. This is in line with current guidelines which recommend that patients with severe congenital or acquired panhypopituitarism with three or more pituitary hormone deficiencies or identified genetic mutations may not require re-evaluation of their GH status; otherwise all patients with CO-GHD require biochemical re-evaluation and reconfirmation of GHD during transition before reinstituting adult GH replacement therapy (200,362). However, of the 34 patients who continued adult rhGH without formal retesting, nine had structural abnormalities on MRI probably were at high risk of ongoing GHD, but three had unexplained GHD and probably should have been retested. Furthermore, some centres still retested those with a high likelihood of permanent GHD, by checking their IGF1 levels, although all were reconfirmed GHD and resumed rhGH. On these grounds, it seems that no clear consensus has been reached as to

whether or not to withdraw treatment and retest those at high risk of permanent GHD. It is also unclear whether those who continued rhGH without biochemical re-testing were re-evaluated at a later stage. For those who restarted rhGH, according to the current guidelines, at completion of somatic growth (approximately 25-30 years old) further re-evaluation should be undertaken with the offer of adult GH replacement therapy and monitoring in accordance with National Institute for Health and Care Excellence guidance (NICE) (TA 64 August 2003).

3.7 Conclusion

In conclusion, this study not only provided a snapshot of the differences in management of CO-GHD during transition across Scotland, but it has also enabled us to identify areas of uncertainty despite there being clinical practice recommendations. Our data showed a substantial proportion of patients with CO-GHD remain GH deficient and most opt for rhGH as adults, although not all patients may require re-evaluation of their GH axis. This study also raises concerns about follow up of those who no longer have GHD and patients with GHD who opted not to resume adult rhGH. The optimal management of adolescents with CO-GHD requires continuous follow up during transition and effective communication between paediatric and adult services.

CHAPTER 4

Bone Mass and Body Composition in Adolescents with Childhood Onset-Growth Hormone Deficiency at Final Height

4.1 Abstract

Background: Childhood onset growth hormone deficiency (CO-GHD) may contribute to low bone density and osteoporosis in adulthood. Data on bone mass and body composition in GH-treated adolescents with CO-GHD at final height are inconsistent.

Aims: To compare size/height corrected parameters of bone mass and body composition in CO-GHD adolescents with healthy controls.

Method: Review of CO-GHD treated with recombinant human growth hormone (rhGH) and who had attained final height between 2005 to 2013 in a single tertiary paediatric centre.

Results: DXA scan results of 21 adolescents with CO-GHD [12 males, (6 with isolated GHD)] were compared to 21 age/height matched controls. The CO-GHD were diagnosed at age 9.4yrs(1.2, 14.5) and first treated with GH at age 10.1 yrs(1.3,14.7) with median duration of treatment 5.6 yrs(2.0, 16.3). Lower TB- BMC for height SDS and for bone area SDS was seen in CO-GHD adolescents, which was pronounced in males but not in females. BMAD SDS of CO-GHD subjects were also significantly lower CO-GHD females, but not in males, compared to controls. Furthermore, subjects with CO-GHD have lower LM for height and higher FM for height compared to controls, and this was more pronounced in males than females (p=0.04). Neither bone nor body composition parameters were correlated with stimulated peak GH /IGF-1 levels at retesting, or with duration of childhood GH treatment or duration of discontinuation of treatment for all patients, or either gender. In this cohort, LM, but not FM, showed significant correlations with TB/LS bone mass.

Conclusion: Males with CO-GHD unlike females have lower TB bone mineralisation for their size and height whereas CO-GHD females appeared to have less mineralisation at the LS. These findings indicate that adolescents with CO-GHD have a low bone mass, despite prior long term rhGH replacement therapy.

4.2 Introduction

Childhood onset GH deficiency (CO-GHD) may contribute to low bone density and osteoporosis in adulthood (92,263). Dual-energy X-ray absorptiometry (DXA), the most commonly used technique to measure bone density and body composition, is subject to the confounding effect of body size, body composition and pubertal maturation in normal growing children as well as children with chronic disease such as GHD (374-376). Recently, the International Society for Clinical Densitometry (ISCD) made recommendations based on reviews of several approaches and methods of the scientific literature that allow adjustment of DXA derivatives (83). Although statements from this and other expert panels have highly recommended to adjust DXA results for height (377), bone size (378-380) and body composition (381), there is still no agreement to what is the optimal approach for correction and interpretation of DXA parameters in clinical practice.

In line with this, clinical studies thus far demonstrate conflicting results regarding bone mass of adolescents with CO- GHD who received childhood recombinant human growth hormone (rhGH) at time of final height. Studies have shown adolescents with CO- GHD to have either normal (223,224) or lower bone mass when compared to the normal population (233,365,382,383). These reports however, have not considered the bone size and height of CO-GHD subjects and no size/height correction was made on the reported data.

This study therefore aims to apply different models of DXA adjustment looking for bone and body composition of GH treated adolescents with CO-GHD after attainment of final height.

4.3 Study Subjects and Methods

4.3.1 Subjects

CO-GHD patients treated with rhGH who had attained final height between 2005 to 2013 at the Royal Hospital for Children, Glasgow were reviewed retrospectively. Eligibility criteria included: fulfilled the clinical and diagnostic criteria for CO-GHD (childhood stimulation GH peak <6.6 µg/l), treated with rhGH during childhood for more than one year before attainment of final height, had attained final height between 2005 -2013 (height velocity <2cm/year), and had DXA scanning of total body (TB) and lumbar spine (LS) as part of their clinical management and re-evaluation at final height and before recommencing adult rhGH. The exclusion criteria were untreated CO-GHD, short duration of childhood rhGH(< one year) or who have not yet attained final height and individuals receiving rhGH for other indications (i.e. Turner syndrome, small for gestational age, SHOX gene haploinsufficiency, and chronic renal insufficiency). All data were collected retrospectively from patients' notes.

4.3.2 Controls

A total of 21 height, age and sex matched healthy controls to the patients were selected from healthy children who had participated in a research project to collect local reference data for bone mass and body composition. All control children underwent TB/ LS-DXA, but no biochemical tests were performed. Pubertal stage was tanner stage 4 to 5 in all males and females in the selected controls, which means they were at or near their final height.

4.3.3 Anthropometry

Weight and height were measured at time of the scan for both patients and controls. Weight, height and BMI were converted to SDS scores using British 1990 reference data (384).

4.3.4 DXA parameters

DXA scans were performed at TB, LS using a narrow fan beam lunar prodigy densitometer (GE Medical Systems, Waukesha, Wisconsin, U.S.A) using the Encore software (Version 8.80.001). Lunar software calculated an ethnicity-, age- and gender-matched bone mineral density (BMD) Z scores using a reference population of over 2,000 US children between the ages of 5 years and 19 years. DXA outcomes were adjusted using the following methods:

BMD Height age Z-score; Height age is at which a child is at 50th percentile for height on growth chart. Children whose height was greater than the median value for height 18years were assigned a height age at 18. BMD Height age Z score for TB, LS were generated by completes software –USA reference ranges.

LS-bone mineral apparent density (BMAD) was calculated as = BMDareal x (4/(π x (mean of L2+L4 width) (378). BMAD SDS was calculated according to age- and sex-matched reference values from the Dutch population (385).

TB, LS- bone area for age and bone mineral content (BMC) for bone area SDSs were calculated according to age- and sex-matched reference values from the Dutch population (385).

The relationship between LM and BMC was estimated according to Crabtree et al (381).

SDSs of the ratio of BMC for height, bone area for height, lean mass (LM) for height, BMC for LM and fat mass (FM) for height were calculated from local Glasgow reference data by dividing the difference of index from the mean of matched height reference on SD [(value – the mean for height)/SD].

4.4 Statistical Analysis

All data were analysed using Minitab software version 17.1. Analyses were performed for whole cohort and separately for males and females. Data are expressed as medians (range) and mean ± SD. Mann-Whitney non-parametric tests were used to examine the differences between groups. Fisher's exact test used to compare categorical data. Spearman rank correlations were used to compare any association between variables. Multiple linear regression models were used to estimate the regression coefficients for LS- BMAD, TB-BMC as dependent variables according to gender and TB and LS bone area, LM, FM, and android (trunk) /gynoid (legs) (A/G) fat ratio as independent variables after adjusting for confounders of age, height, and BMI at final height. All graphs were prepared using GraphPad Prism 6 software (GraphPad Software, San Diego, CA, USA). P value of < 0.05 was considered significant. This is a retrospective review of CO-GHD treated with recombinant human growth hormone (rhGH) and who had attained final height between 2005 to 2013 in a single tertiary paediatric centre, no sample size and power calculation were required.

4.5 Results

4.5.1 General characteristics

A total of 70 patients were identified, only 21(12 males, 9 females) who met inclusion criteria were included, Figure 4-1.

CO-GHD was diagnosed at median (range) age 9.5 years (1.2, 14.5) and rhGH started during childhood at age 10.1 years (1.3, 14.7) with median duration of treatment of 5.6 years (2.0, 16.3). Out of 21 CO-GHD subjects, 12 (57%) had a congenital form of GHD, (10/12 (48%) a structural hypothalamic-pituitary abnormality and idiopathic GHD in two (9%)), while the remaining 9/21 (43%) had acquired GHD secondary to tumour or tumour related cranial irradiation.

Six patients of the 21(29%) (3 males, 3 females) had isolated GHD, four had a structural hypothalamic-pituitary abnormality on MRI scan and two had idiopathic GHD.

15 of the 21 (71%) (9 males, 6 females) had multiple pituitary hormone deficiencies (MPHD) (n=8 had one additional pituitary hormone deficiency (PHD), n=3 with two, n=1 with three and n=3 with four additional PHDs (panhypopituitrism)). Of these, six had a structural hypothalamic-pituitary abnormality on MRI scan and 9 were secondary due to tumour or tumour related cranial irradiation. All the MPHD subjects were on hormonal replacement where necessary (glucocorticoid (n=6), T4 (n=9), sex steroid (n=10), and desmopressin (n=4)) and all were adequately controlled.

All patients ceased rhGH treatment at final height and their DXA scans were performed at a median of 0.6years (0.0, 2.0) after stopping rhGH. After retesting, 19/21 of them were confirmed with persistent GHD, whereas two patients with IGHD (idiopathic and ectopic posterior pituitary) were no longer GH deficient on retesting

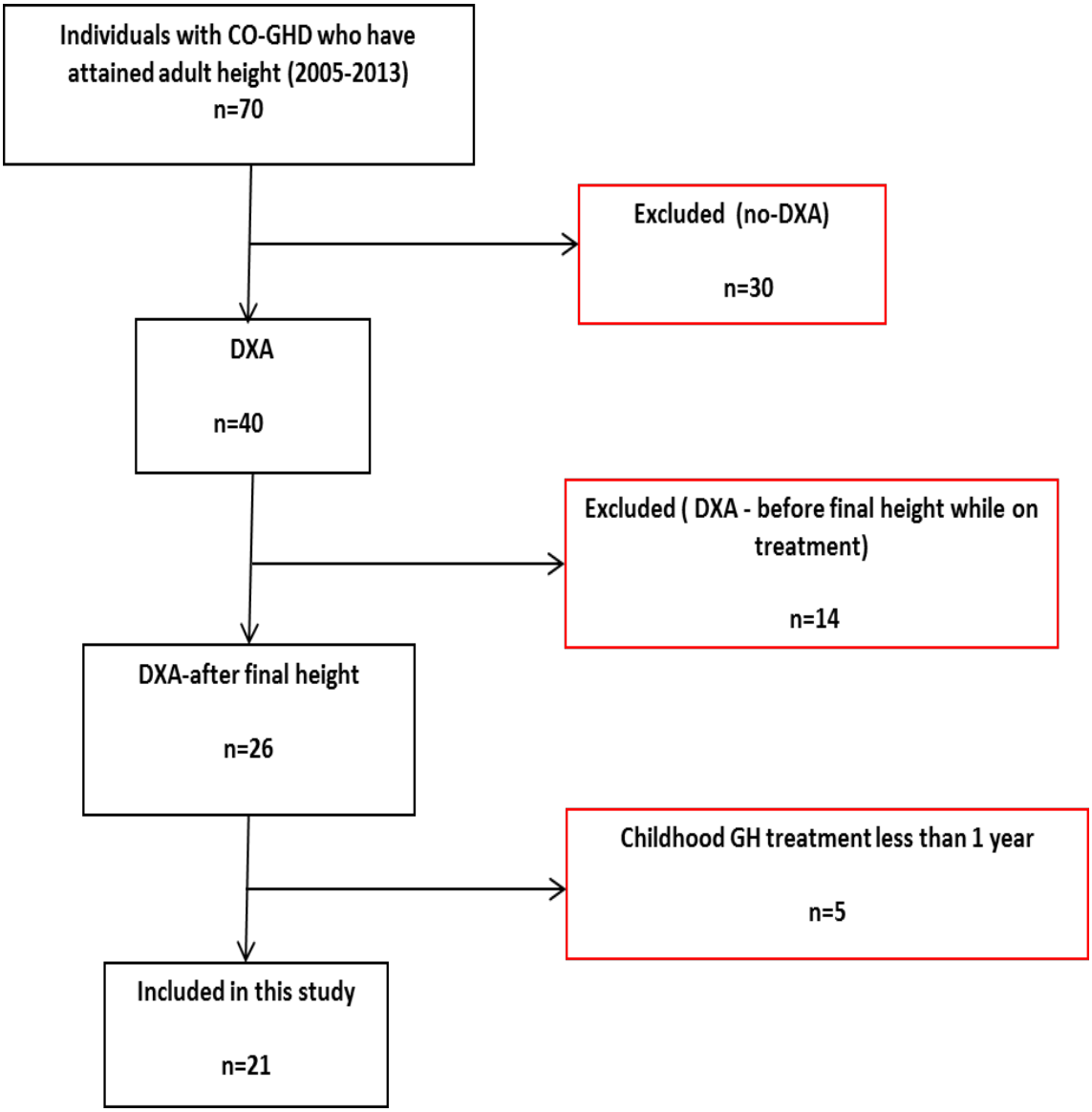


Figure 4-1 Study cohort flow chart

4.5.2 Anthropometric characteristics

Anthropometric characteristics are summarized in (Table 4-1, Figure 4-2). For all subjects combined and separately, age, height SDS, weight SDS and BMI SDS were similar in patients with CO-GHD and controls. Of the 21 patients with CO-GHD, four patients (19%) (Three males- one female) were obese (BMI SDS > + 2; 3.4, 2.9, 3.6, 2.6 respectively) and among the control group, only one male was obese (BMI SDS 2.9).

Table 4-1 Anthropometric characteristics of patients with CO-GHD and controls.

	CO-GHD (n=21)	**Controls (n=21)**	**P-value* (95%CI)**
	Median (range)	**Median (range)**	
Sex m:f	12:9	12:9	-
Age(yrs)			
All	17.0 (14.9, 19.5)	16.9 (14.8, 19.0)	0.31(-0.38,1.43)
Males	17.6 (15.2, 19.2)	17.0 (15.4, 18.5)	0.34(-0.30,1.30)
Females	16.6 (14.9, 19.5)	16.2 (14.8, 19.0)	0.53 (-2.13,1.04)
Height SDS			
All	-0.9 (-1.9, 1.2)	-0.3 (-2.3, 1.3)	0.16(-0.80,0.23)
Males	-0.7 (-1.9, 1.2)	-0.3 (-1.4, 1.2)	0.50(-0.60,1.06)
Females	-1.0 (-1.6, 0.5)	-0.7 (-2.3, 0.2)	0.18(-0.31,0.92)
Weight SDS			
All	0.2 (-2.3, 4.2)	-0.1 (-1.4 - 2.2)	0.49(-0.61,1.13)
Males	0.1 (-2.3, 4.2)	0.1 (-0.8 - 2.2)	0.93(-1.41,1.33)
Females	0.3 (-1.8, 2.1)	-0.4 (-1.4 - 1.0)	0.2 (-1.83,0.70)
BMI SDS			
All	0.7 (-1.8, 3.6)	0.4 (-1.5, 2.9)	0.23(-0.30,1.30)
Males	0.5 (-1.8, 3.6)	0.5 (-1.3, 2.9)	0.79(-1.79,1.04)
Females	1.1 (-1.3, 2.6)	0.0 (-1.5, 1.2)	0.09(-2.05,0.19)

Data are presented as median and ranges are given in parentheses

* Mann-Whitney tests p value (95% confidence interval)

4.5.3 Bone measures at TB and LS

Median and ranges of TB and LS measurements of patients with CO-GHD compared with control data are reported in (Table 4-2, 4-3), and individual data is shown graphically in (Figure 4-2, 4-3). BMD-Z-score $_{\text{height-age}}$ at both sites TB/LS did not differ significantly between males and females with CO-GHD and their controls (all within the range of$\pm$2 SD of age). Lower TB-BMC for both bone area and height SDSs were seen in CO-GHD and that was pronounced in males but not in females, Table 4-2.

BMAD SDS of CO-GHD subjects were also significantly lower than controls, with approximately one quarter of CO-GHD (n=5/21 (24%)) having reduced LS-BMAD SDS < -1.5 SDS, but none below – 2 SDS. These were three males (Ectopic posterior pituitary- Septo-optic dysplasia with hypopituitarism –Idiopathic isolated GHD (Charge syndrome)) and two females (Idiopathic isolated GHD- Craniopharyngioma).

Table 4-2 Parameters of bone density at total body.

	CO-GHD (n=21)		Controls (n=21)		P-value* (95%CI)
	Median (range)	**Mean (SD)**	**Median (range)**	**Mean (SD)**	
TB-BMD (g/cm^2)					
All	1.04 (0.97, 1.31)	1.06 (0.09)	1.16(1.04, 1.30)	1.15(0.08)	**<0.01(-0.15, -0.04)**
Males	1.04 (0.97, 1.31)	1.08 (0.10)	1.20(1.11, 1.30)	1.19(0.06)	**0.01(0.03, 0.20)**
Females	1.01(0.97, 1.09)	1.02 (0.04)	1.06 (1.04, 1.21)	1.09(0.06)	**0.04 (0.01, 0.12)**
TB-BMD Z-score $_{age}$					
All	-1.40 (-2.50, 1.20)	-1.08(1.04)	-0.30 (-1.40, 1.20)	0.03(0.84)	**<0.01(-1.70, -0.50)**
Males	-1.10 (-2.50, 1.20)	-0.95(1.23)	0.20 (-1.40, 1.20)	0.25(0.83)	**0.01 (0.20, 2.30)**
Females	-1.4 (-2.20, -0.20)	-1.25(0.75)	-0.60 (-0.90, 1.10)	-0.26(0.79)	**0.04 (0.01, 1.60)**
TB-BMD Z-score $_{height-age}$					
All	-0.15(-1.80, 2.10)	-0.07(1.01)	0.50 (-1.40, 2.60)	0.52(1.03)	0.05 (-1.40, 0.00)
Males	-0.40 (-1.80, 2.10)	-0.18(1.22)	0.65(-1.40, 2.60)	0.47(1.19)	0.16 (-0.40,1.80)
Females	-0.10 (-0.90, 1.30)	0.05(0.72)	0.2(-0.30, 1.90)	0.60(0.83)	0.18 (-0.40, 1.40)
TB-BMC(g)					
All	2055.9 (1721.9, 4008.8)	2252 (557)	2565.2 (1824.0, 3412.7)	2552 (452)	**0.01(-677.0, -61.6)**
Males	2509.0 (1832.5, 4008.8)	2542 (608)	2903.9 (2452.7, 3412.7)	2866.1(295.6)	0.06 (-44.6, 789.3)
Females	1865.2 (1721.9, 2162.8)	1897.9(138.8)	2143.6 (1824.0, 2767.1)	2168.6(275.0)	**0.01 (43.6, 462.0)**
TB-BMC for BA SDS					
All	0.05(-0.60, 0.70)	-0.01(0.40)	0.16 (-0.15, 1.10)	0.33(0.33)	**0.01(-0.59, -0.09)**
Males	-0.20(-0.60, 0.70)	-0.03(0.46)	0.55(-0.10, 1.10)	0.43(0.35)	**0.02 (0.06, 0.90)**
Females	0.1 (-0.50, 0.50)	0.02(0.34)	0.1(-0.10, 0.70)	0.20(0.28)	0.68 (-0.12,0.59)
BMC for height SDS					
All	-0.03(-2.84, 2.15)	-0.18(1.06)	0.45(-1.46, 1.91)	0.45(0.86)	**0.03 (-1.19, -0.02)**
Males	-0.45(-2.84, 2.15)	-0.47(1.30)	0.41(-1.46, 1.66)	0.35(0.78)	**0.04 (0.07, 1.68)**
Females	0.21(-1.11, 0.71)	0.15(0.54)	0.73(-0.97, 1.91)	0.57(0.99)	0.33 (-0.57,1.32)
TB- Bone area (cm^2)					
All	2013.0 (1710.0, 3060.0)	2089.7(338.8)	2226.5(1753.0, 2694.0)	2203.5(267.9)	0.12 (-330.9, 54.0)
Males	2282.0 (1881.0, 3060.0)	2295.2(321.7)	2378.0(2080.0, 2694.0)	2387.4(184.9)	0.25 (-88.9, 331.0)
Females	1823.0 (1710.0, 2025.0)	1838.4(119.3)	1940.0(1753.0, 2277.0)	1978.8(156.2)	0.05 (-9.0, 282.0)
Bone area for height SDS					
All	0.08 (-2.26, 3.23)	0.13(1.27)	0.46(-1.49, 1.67)	0.36(0.90)	0.28 (-1.01,0.38)
Males	-0.47(-2.26, 3.23)	0.08(1.64)	0.40 (-1.49, 1.50)	0.30(0.97)	0.47 (-1.02,1.67)
Females	0.32(-1.46, 1.20)	0.19(0.71)	0.75 (-1.01, 1.67)	0.43(0.86)	0.72 (-0.71,1.01)

The data are presented as median (range) and mean (standard deviation)

* Mann-Whitney tests p value (95% confidence interval)

Table 4-3 Parameters of bone density at lumber spine.

	CO-GHD (n=21)		Controls (n=21)		P-value* (95% CI)
	Median (ranges)	**Mean (SD)**	**Median (ranges)**	**Mean (SD)**	
LS-BMD (g/cm2)					
All	1.01(0.88, 1.39)	1.05(0.13)	1.12(0.95, 1.47)	1.14(0.11)	**0.01 (-0.15, -0.03)**
Males	1.11(0.88, 1.39)	1.10(0.15)	1.15(0.95, 1.3)	1.15(0.09)	0.58 (-0.06,0.18)
Females	0.99(0.91, 1.11)	0.99(0.05)	1.09 (1.03, 1.47)	1.14(0.13)	**<0.01 (0.05, 0.20)**
LS-BMD Z-score $_{age}$					
All	-1.40(-2.40, 1.10)	-1.13(1.08)	-0.40(-2.90, 2.80)	-0.51(1.23)	0.07 (-1.30, 0.10)
Males	-0.95(-2.40, 1.10)	-0.82(1.20)	-0.35(-2.90, 1.0)	-0.61(1.09)	0.62 (-0.79,1.40)
Females	-1.80(-2.30, 1.10)	-1.55(0.78)	-1.10(-1.70, 2.80)	-0.37(1.45)	**0.04 (0.01, 2.10)**
LS-BMD Z-score $_{height-age}$					
All	-0.30(-1.30, 1.30)	-0.09(0.90)	0.20(-2.90, 3.00)	0.24(1.14)	0.16 (-0.90, 0.20)
Males	-0.15(-1.30, 1.30)	0.05(1.11)	0.15(-2.90, 1.00)	-0.14(0.99)	0.90 (-1.10, 0.90)
Females	-0.30 (-1.10, 0.40)	-0.28(0.51)	0.20(-0.30, 3.00)	0.76(1.18)	**0.04 (0.01, 2.20)**
LS-BMC(g)					
All	37.50 (28.13, 66.60)	41.52(9.93)	49.0(37.26, 64.47)	49.52(7.84)	**<0.01(-14.92, -3.51)**
Males	46.20 (35.6, 66.60)	49.66(12.19)	53.37(43.3, 60.6)	53.23(5.18)	0.28 (-3.97,13.40)
Females	34.34(28.13, 44.11)	34.80(4.67)	42.85(37.26, 64.47)	44.57(8.27)	**<0.01 (3.81, 14.07)**
LS-BMC for BA SDS					
All	0.00(-1.00, 1.20)	0.03(0.66)	0.13(-0.70, 1.30)	0.18(0.51)	0.32 (-0.55, 0.19)
Males	0.05(-1.00, 1.20)	0.07(0.75)	0.16 (-0.70, 1.20)	0.22(0.46)	0.49 (-0.57,0.80)
Females	-0.10(-0.70, 1.10)	-0.01(0.55)	-0.10(-0.50, 1.30)	0.14(0.59)	0.69 (-0.40,0.75)
LS-Width					
All	3.90(3.40, 4.70)	3.98(0.39)	4.20(3.40, 4.60)	4.16(0.34)	0.13(-0.50,0.10)
Males	4.30(3.70, 4.70)	4.22(0.32)	4.40(4.00, 4.60)	4.35(0.18)	0.34 (-0.10,0.40)
Females	3.60(3.40, 4.20)	3.66(0.23)	3.90(3.40, 4.50)	3.90(0.32)	0.10 (-0.10,0.50)
LS-BMAD (g/cm^3)					
All	0.35(0.29, 0.37)	0.33(0.02)	0.36(0.30, 0.44)	0.36(0.03)	0.03 (-0.04,-0.00)
Males	0.33(0.29, 0.37)	0.32(0.03)	0.34(0.30, 0.42)	0.34(0.03)	0.26 (-0.01,0.04)
Females	0.35(0.30, 0.37)	0.34(0.02)	0.38(0.31, 0.44)	0.38(0.03)	**0.01 (0.01, 0.07)**
LS-BMAD SDS $_{age}$					
All	-0.91(-2.03, 0.99)	-0.67(0.87)	0.02(-1.90, 1.90)	0.03(0.90)	**0.01(-1.28,-0.17)**
Males	-0.67(-2.0, 1.0)	-0.44(1.06)	0.12 (-1.4, 1.9)	0.17(0.88)	0.26 (-0.44,1.51)
Females	-1.0(-1.7, -0.5)	-0.97(0.42)	-0.5(-1.9, 1.1)	-0.14(0.95)	**0.04 (0.03,1.71)**

The data are presented as median (range) and mean (standard deviation)

* Mann-Whitney tests p value (95% confidence interval)

LS: lumbar spine; BMD: bone mineral density; BMC: bone mineral content; BMAD: bone mineral apparent density

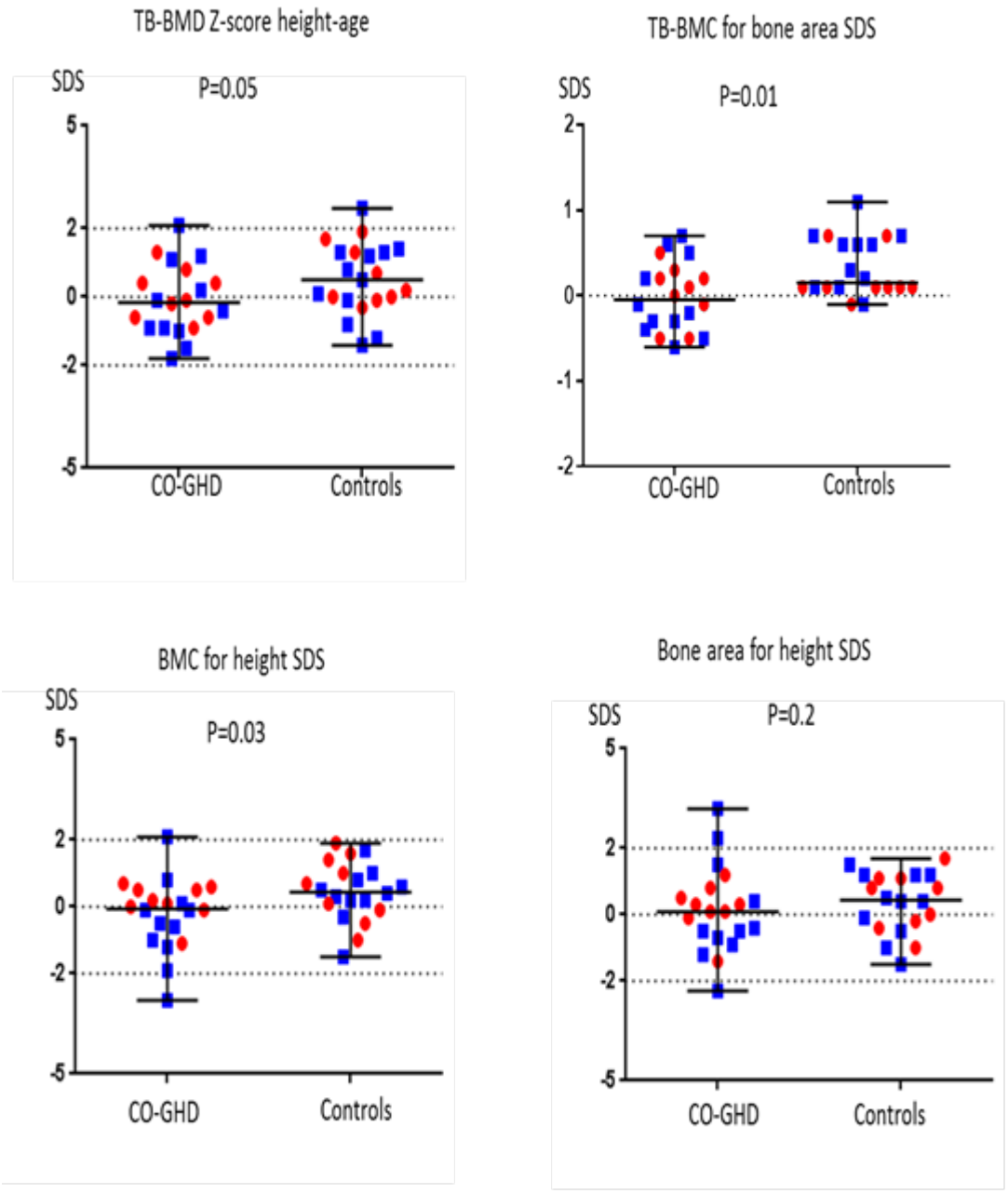


Figure 4-2 Individual values (median-range) of total body bone mass parameters of patients with CO-GHD at final height and controls.
(Blue Square, Males; Red Circle, Females).

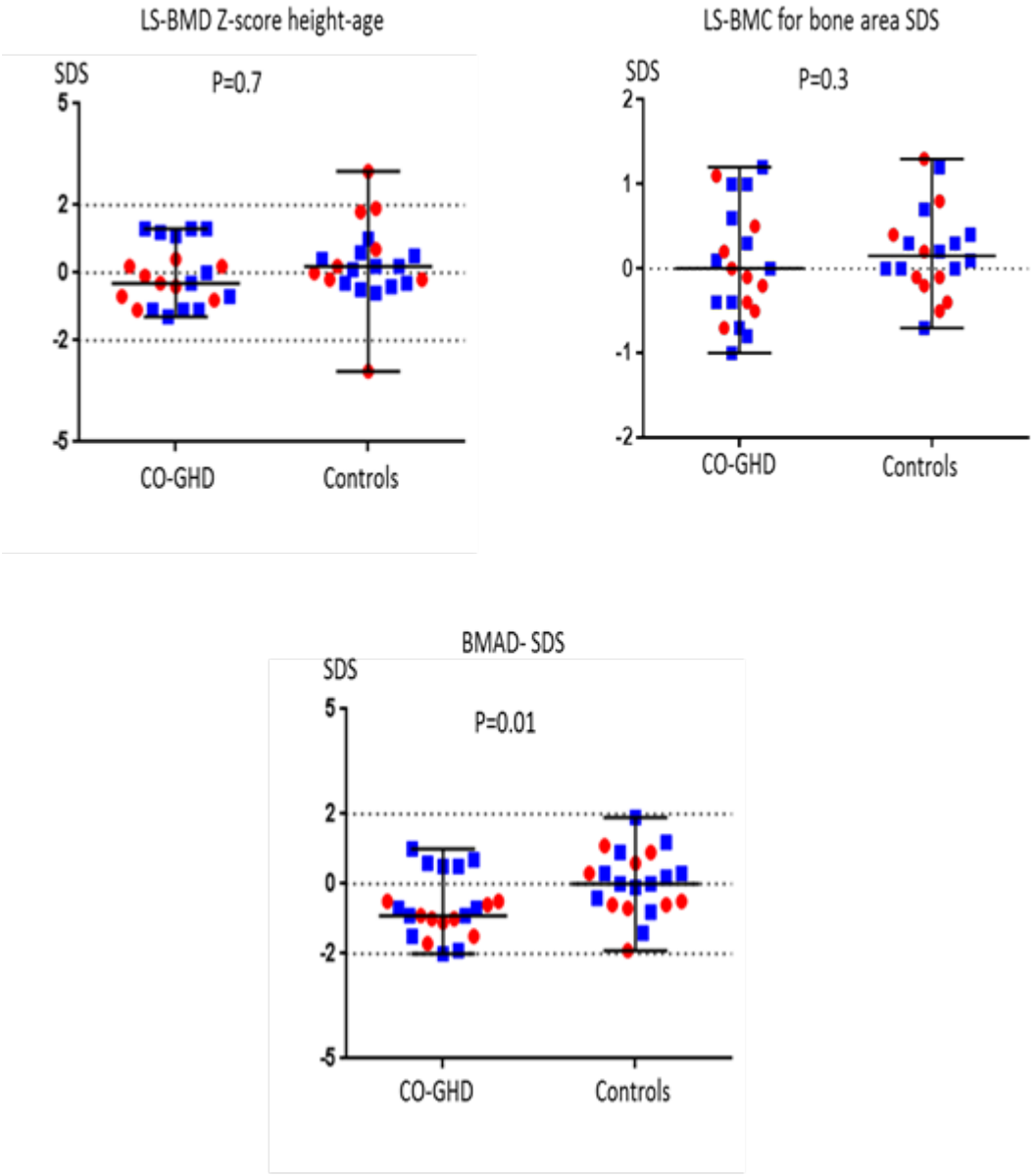


Figure 4-3 Individual values (median-range) of lumbar spine bone density parameters of patients with CO-GHD at final height and controls.
(Blue Square, Males; Red Circle, Females

4.5.4 Body composition

From (Table 4-4 and Figure 4-4), subjects with CO-GHD had significant reduced LM for height (index of primary muscle defect “Sarcopenia”) [-0.63 (-3.00, 1.87) CO-GHD vs. 0.29 (-1.75, 1.97) controls, p=0.04] with one girl (GHD with hypogonadism) having LM for height SDS below -2.

CO-GHD had higher FM for height compared to controls [1.01 (-0.65, 6.92) CO-GHD vs. -0.32 (-1.15, 3.25) controls, p<0.01], and this was more obvious in males than females (three males (two septo-optic dysplasia/hypopituitarism and one patient craniopharyngioma) and one female (craniopharyngioma) having extremely high FM for height SDS >5).

The median SDS of BMC for LM (index of primary bone defect “Osteopenia”) in CO-GHD was comparable to control subjects (p =0.67) with only two patients (males) (10%) having BMC for LM SDS less than -2 (craniopharyngioma, idiopathic IGHD).

The median of A/G ratio was also significantly higher in CO-GHD compared to controls (1.10 (0.55, 1.24) vs. 0.59 (0.53, 1.45) respectively, p<0.01).

Table 4-4 Body composition parameters of patients with CO-GHD at final height and controls.

	CO-GHD (n=21)		Controls (n=21)		P-value* (95% CI)
	Median (ranges)	**Mean (SD)**	**Median (ranges)**	**Mean (SD)**	
LM (kg)					
All	38.20(26.10, 62.31)	40.79(10.57)	49.0(29.8, 60.3)	46.34(10.52)	0.13 (-13.26,1.15)
Males	47.1 (36.5, 62.3)	47.74(8.68)	53.8 (46.9, 60.4)	54.04(4.82)	0.06 (-0.27,13.11)
Females	30.0(26.1, 39.0)	32.29(4.86)	34.9(29.8, 48.1)	36.08(6.09)	0.21(-2.63, 8.81)
LM for height SDS					
All	-0.63 (-3.00, 1.87)	-0.47(1.24)	0.29 (-1.75, 1.97)	0.24 (1.06)	**0.04 (-1.52, -0.01)**
Males	-0.39 (-1.57, 1.87)	-0.21(0.98)	0.39 (-1.31, 1.89)	0.30 (0.96)	0.16 (-0.29, 1.33)
Females	-0.87 (-3.00, 1.71)	-0.78(1.50)	0.29 (-1.75, 1.97)	0.17(1.24)	0.18 (-0.58, 2.44)
BMC for LM SDS					
All	0.56 (-4.20, 5.65)	0.54(2.13)	0.5 (-1.51, 1.96)	0.32(0.95)	0.67(-0.50, 0.94)
Males	0.23 (-4.20, 5.65)	0.47(2.84)	0.18 (-1.52, 1.96)	0.10(0.98)	0.64 (-1.73,1.28)
Females	0.63 (-0.92, 2.61)	0.62(0.84)	0.62 (-2.51, 1.82)	0.58(0.88)	0.92 (-0.93, 0.76)
FM (kg)					
All	21.35 (4.59, 70.78)	26.04(17.60)	10.11(5.66, 48.10)	14.45(10.22)	**<0.01(2.86, 15.51)**
Males	16.27 (4.59, 70.78)	26.78(22.82)	7.76 (5.66, 32.52)	11.03(7.85)	**0.02 (-31.29, -1.58)**
Females	23.67 (14.54, 44.94)	25.15(9.14)	16.22 (9.31, 48.10)	19.01(11.65)	0.07 (-14.72, 0.34)
FM for height SDS					
All	1.01(-0.65, 6.29)	1.92(2.25)	-0.32 (-1.15, 3.25)	0.14(1.14)	**<0.01 (0.43, 2.29)**
Males	0.98 (-0.65, 6.29)	1.87(2.68)	-0.47 (-1.15, 2.61)	-0.12(1.03)	**0.02 (-3.68, -0.18)**
Females	1.05 (0.41, 5.77)	1.97(1.74)	0.52 (-0.56, 3.25)	0.51(1.23)	0.05 (-2.70, 0.01)
A/G fat ratio					
ALL	1.10(0.55, 1.24)	1.03(0.19)	0.59 (0.53, 1.45)	0.76(0.23)	**<0.01 (0.17, 0.46)**
Males	1.14 (0.55, 1.24)	1.04(0.23)	0.69 (0.53, 1.45)	0.80(0.28)	0.07 (-0.51, 0.03)
Females	1.03 (0.71, 1.24)	1.01(0.14)	0.68 (0.53, 0.98)	0.71(0.16)	**<0.01 (-0.47, -0.13)**

The data are presented as median (range) and mean (standard deviation)

* Mann-Whitney tests p value (95% confidence interval)

LM: lean mass; FM: fat mass; A/G: Android/Gynoid fat ratio

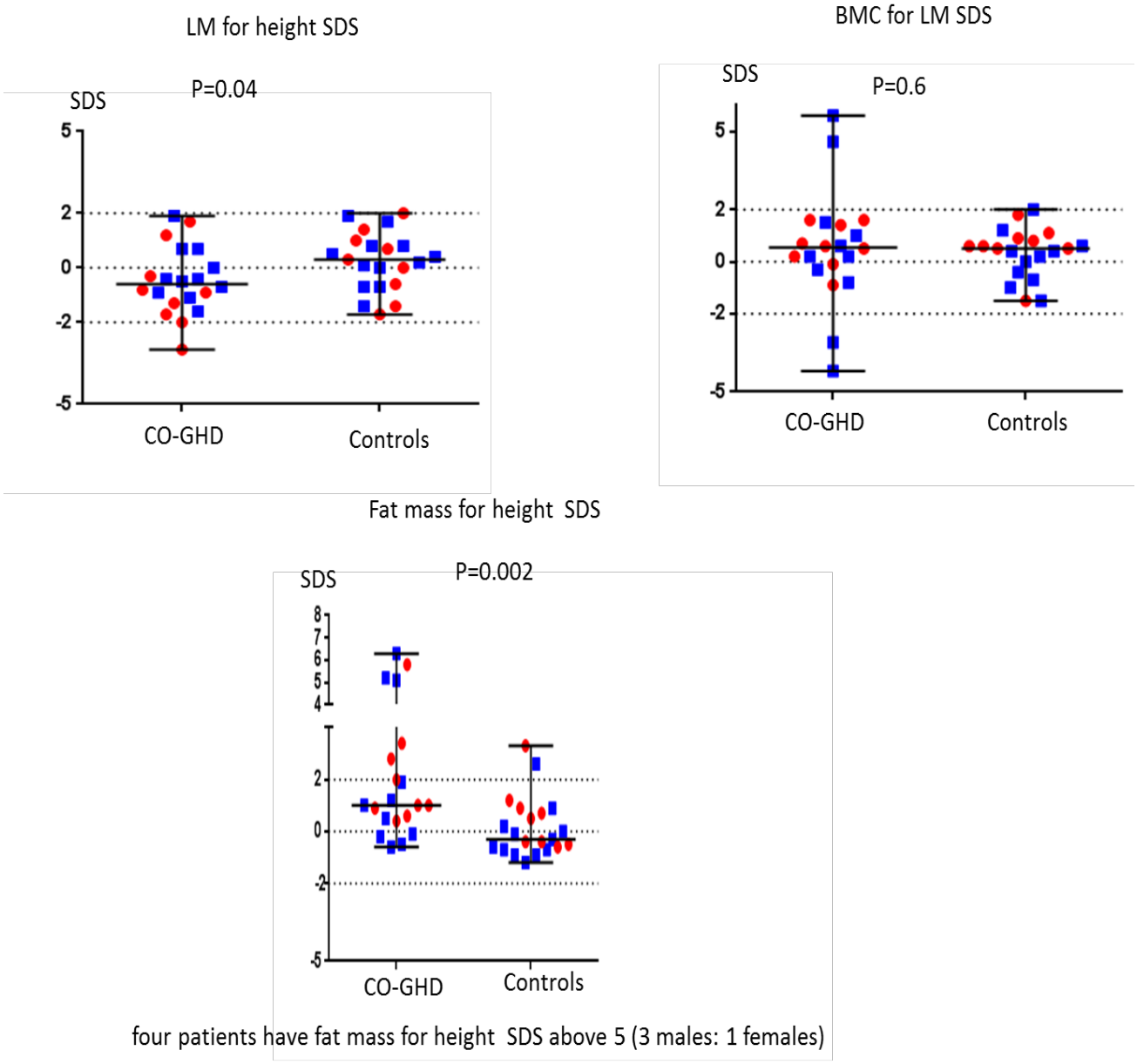


Figure 4-4 Individual values of body composition parameters of patients with CO-GHD and controls.
(Blue Square, Males; Red Circle, Females)

4.5.5 DXA parameter in relation to disease condition

Biochemical data was similar in the group of patients who had congenital GHD (total n=12, 7 males, 5 IGHD: 7 MPHD) to those with acquired GHD (n=9, 5 males, 1 IGHD: 8 MPHD) with no differences in the time of diagnosis and duration of childhood rhGH treatment, Table 4-5.
Lower TB and LS (p=0.01) -BMC for bone area SDS (p= 0.03, 0.01, respectively) in congenital GHD compared to those with acquired GHD (Figure 4-5). These differences existed in subgroups analysis of those with IGHD (n=6) to those with MPHD (n=15) as patients with IGHD had lower BMC for bone area SDS significantly at LS (P=0.04), but not significant at TB (p= 0.31) compared to those with MPHD.

Table 4-5 Clinical and anthropometrics of patients with congenital GHD, and acquired GHD.

	Congenital (n=12)	**Acquired (n=9)**	**P-value* (95%CI)**
Gender (m/f)	7/5	5/4	0.9
Age	17.7 (15.9, 19.5)	16.5 (14.9, 18.5)	0.09 (-0.30,2.39)
Age of diagnosis	7.9 (1.2, 14.2)	9.5 (4.6, 14.3)	0.65 (-5.94,3.09)
Age of starting rhGH therapy	10.5 (1.3, 14.7)	9.9 (7.1, 14.3)	0.54 (-6.65,3.33)
Duration of rhGH treatment	5.0 (3.3, 16.3)	4.1 (2.0, 10.8)	0.14 (-1.69,8.49)
Age of stopping rhGH	17.8 (14.7, 19.2)	16.3 (14.4, 18.2)	0.56 (-0.58,1.99)
Height SDS	-0.9 (-1.9, 0.8)	-1.1 (-1.9, 1.2)	0.64 (-0.68,1.02)
Weight SDS	0.0 (-2.4, 3.6)	0.3 (-0.8, 4.2)	0.37 (-2.42,0.92)
BMI SDS	0.3 (-1.8, 3.4)	0.8 (0.4, 3.6)	0.30 (-2.24,0.70)
GH peak on diagnosis	0.5 (0.1, 5.7)	2.1 (1.7, 2.4)	0.18 (-2.30,4.03)
GH peak at retesting	0.5 (0.1, 23.7)	0.5 (0.1, 3.2)	0.62 (-0.29,2.90)
No of additional PHDs(0/1/2/3/4)	(5/5/1/1/2)	(1/3/2/0/1)	-

Data are presented as median and ranges are given in parentheses

*Mann-Whitney tests p value (95% confidence interval)

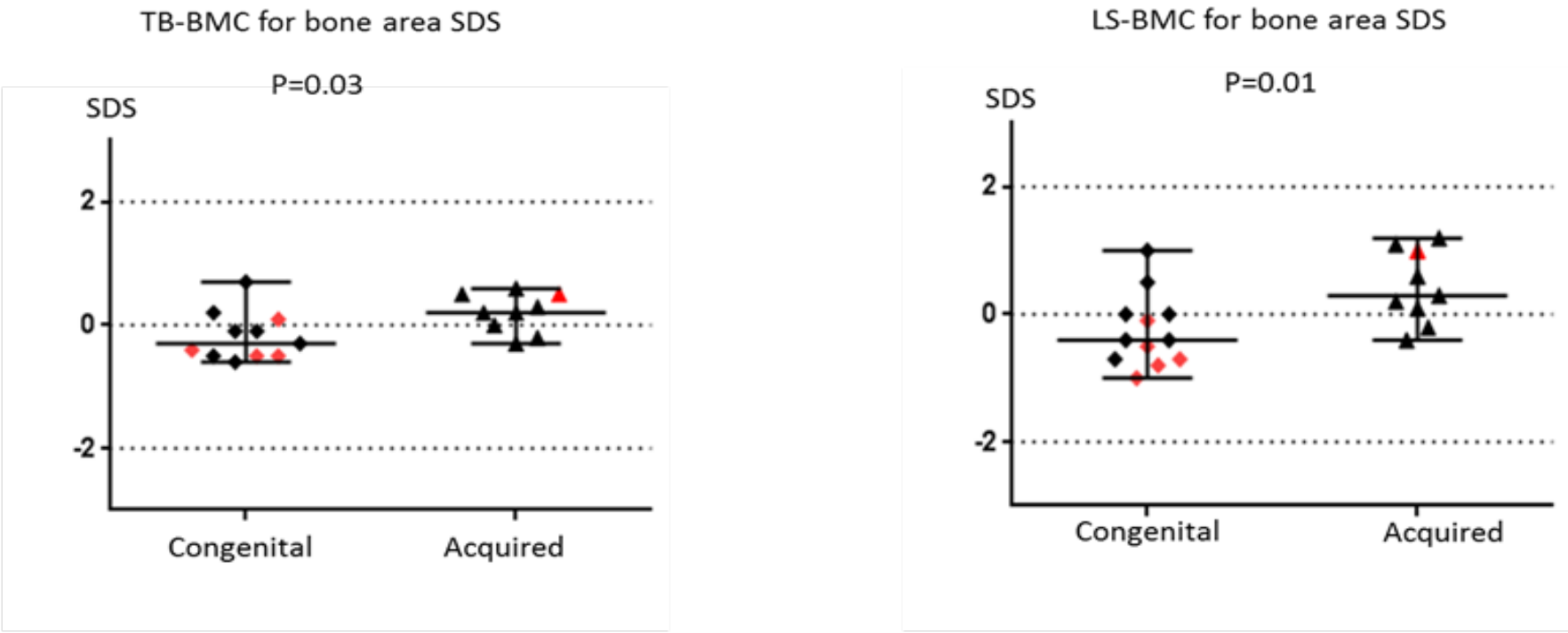


Figure 4-5 Individual data of bone density parameters in groups of congenital GHD vs. acquired GHD.
(Diamond, Congenital GHD; Triangle, Acquired GHD) (Red, IGHD; Black, MPHD).

4.5.6 Correlation of DXA parameters and clinical data

Neither bone at both sites TB/LS nor body composition parameters were correlated with stimulated peak GH /IGF1 levels at retesting or with duration of childhood rhGH treatment.

FM correlated positively with the number of additional PHDs in subjects with GHD (r= 0.52, P = 0.02), and higher FM was more likely in those who were received thyroxine (T4) and hydrocortisone replacement therapy (r=0.51, p= 0.02 T4; r=0.45, p= 0.04 glucocorticoid), Figure 4-6.

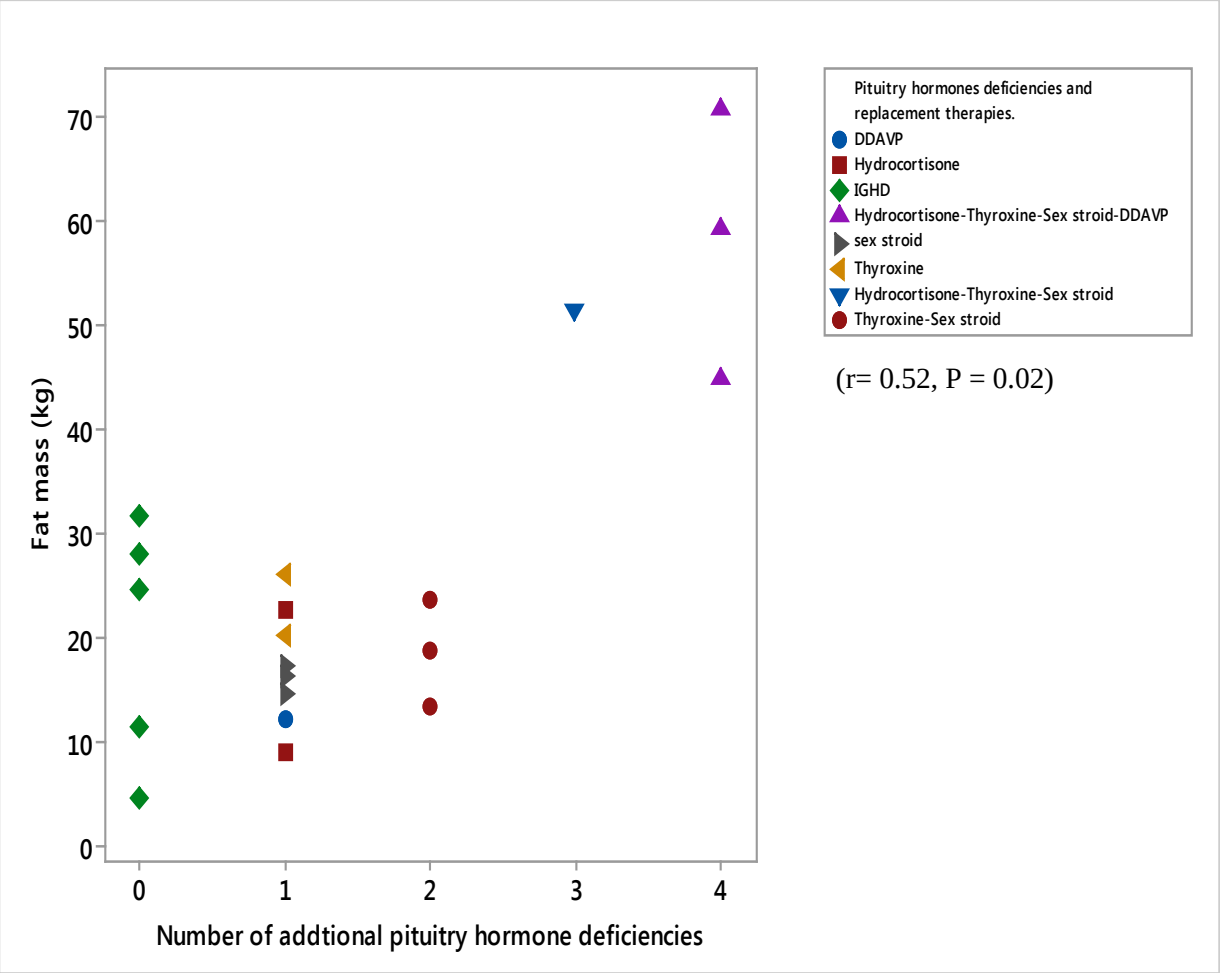


Figure 4-6 Fat mass and number of additional pituitary hormone deficiencies

4.5.7 Relationships between body composition and bone mass

Lean mass had a stronger positive correlation with BMC and BMD at different sites TB/LS as compared to fat mass in both groups. BMI and A/G ratio had a positive correlation with TB-BMC only in CO-GHD group, Table 4-6.

Table 4-6 Spearman correlation between bone density, anthropometric and body composition measures

	TB-BMC	TB-BMD	LS-BMC	LS-BMD	LS-BMAD
Age					
All	0.02, p=0.90	-0.11, p=0.48	-0.09, p=0.53	-0.21, p= 0.17	-0.10, p= 0.50
Cases	-0.07, p= 0.75	-0.29, p= 0.19	-0.06, p=0.78	-0.07, p=0.75	0.03, p=0.88
Controls	0.26, p= 0.25	0.25, p= 0.28	-0.08, p=0.71	-0.36, p= 0.10	-0.22, p= 0.33
Height					
All	**0.74, p<0.01**	**0.46, p=0.02**	**0.65, p<0.01**	**0.41, p<0.01**	-0.17, p=0.26
Cases	**0.75, p<0.01**	0.32, p= 0.14	**0.71, p<0.01**	**0.45, p= 0.03**	-0.16, p= 0.47
controls	**0.76, p<0.01**	**0.56, p=0.01**	**0.55, p<0.01**	0.27, p=0.23	-0.31, p= 0.16
BMI					
All	**0.31, p=0.05**	0.25, p= 0.11	0.12, p=0.42	0.14, p=0.36	-0.09,p=0.57
Cases	**0.45, p=0.04**	0.35, p=0.11	0.25, p=0.25	0.40, p=0.06	0.22,p= 0.33
Controls	0.38, p=0.09	0.36, p=0.11	0.18, p= 0.42	-0.04, p=0.85	-0.23,p=0.30
LM					
All	**0.86, p<0.01**	**0.62, p<0.01**	**0.86, p<0.01**	**0.76, p<0.01**	-0.22, p= 0.1
Cases	**0.83, p<0.01**	**0.47,p= 0.03**	**0.77, p<0.01**	**0.58, p<0.01**	-0.11,p=0.63
Controls	**0.92, p<0.01**	**0.74, p<0.01**	**0.612, p<0.01**	0.28, p= 0.21	-0.45,p= 0.06
FM					
All	-0.09, p=0.54	-0.11, p=0.47	-0.27, p= 0.08	-0.15, p=0.33	0.01, p= 0.91
Cases	0.18, p=0.43	0.08, p=0.72	-0.04, p=0.86	0.07, p= 0.7	0.13, p= 0.56
Controls	-0.17, p=0.47	0.00, p=0.99	-0.26, p= 0.24	-0.28, p=0.20	0.171, p= 0.45
A/G					
All	0.01, p=0.90	-0.03, p=0.81	-0.17, p=0.28	-0.08, p=0.58	-0.23, p=0.14
Cases	**0.46, p= 0.03**	0.43, p=0.05	0.32, p= 0.15	0.38, p=0.09	0.13,p= 0.56
Controls	0.08, p=0.72	0.02, p=0.90	-0.13, p=0.56	-0.31, p= 0.17	-0.32 ,p= 0.16

TB: total body; LS: lumbar spine; BMI: Body Mass Index; BMD: bone mineral density; BMC: bone mineral content; BMAD: bone mineral apparent density; A/G: Android/Gynoid fat ratio

For parameters showing significant correlations with BMC and BMD at both sites, gender specific multiple generalized linear models were constructed to determine their potential independent contributions to TB-BMC and LS-BMAD after adjusting for age, height and BMI among either gender groups.

Multiple linear regression analysis revealed that bone area was the strongest predictor of TB- BMC in both CO-GHD and controls, followed by LM and A/G ration in females as seen in Table 4-7.

No other predictions were observed for both TB-BMC and LS-BMAD in both gender groups of CO-GHD.

Table 4-7 Multiple linear regressions showing the effect of body parameters on TB-BMC and LS-BMAD after adjustment for variables age, height, and BMI

Independent	All population				CO-GHD				Controls			
	Males		Females		Males		Females		Males		Females	
	β	P-value	β	P-value	β	P-value	β	P-value	β	P-value	β	P-value
Bone area												
TB-BMC	1.966	**<0.01**	1.834	**<0.01**	2.041	**<0.01**	0.712	0.45	1.387	**<0.01**	3.022	**<0.01**
LS-BMAD	-0.003	**0.04**	-0.000	0.85	-0.006	0.14	-0.0077	0.08	-0.00	0.69	-0.003	0.48
LM												
TB-BMC	0.025	0.17	0.045	**<0.01**	-0.012	0.82	0.025	0.16	0.021	0.43	0.029	0.31
LS-BMAD	-0.00	0.98	0.000	0.10	0.000	0.82	-0.00	0.91	-0.000	0.25	0.000	0.39
FM												
TB-BMC	-0.030	0.05	0.014	0.13	-0.022	0.61	-0.0174	0.39	-0.0151	0.60	0.014	0.15
LS-BMAD	-0.00	0.81	0.000	0.06	-0.000	0.44	-0.00	0.93	0.000	0.31	0.00	0.11
A/G												
TB-BMC	-146	0.71	-689	**0.03**	427	0.65	312	0.42	-782	**0.04**	-690	0.29
LS-BMAD	0.020	0.63	-0.0819	0.13	0.069	0.24	-0.0388	0.57	-0.0178	0.75	-0.046	0.75

β: regression coefficient; TB: total body; LS: lumbar spine; BMC: total body bone mineral content; BMAD: bone mineral apparent density; LM: lean mass; FM: fat mass; A/G: Android/Gynoid fat ratio

4.6 Discussion

In this study, bone and body composition of adolescents with CO-GHD after final height were compared with normal subjects matched for age, height and sex. Several studies so far have investigated bone and body composition of adolescents with CO-GHD at time of final height yet were inconsistent (233,236,365,382,383). Using DXA corrected for age, height, size and body composition, our analysis showed that adolescents with CO-GHD have normal areal BMD height-age Z-score but lower volumetric LS-BMAD with reduced BMC measures for bone area and height; although most of the values fell within the normal range (± 2 SDS). Our findings are consistent with previous reports of lower bone density and mineralisation in CO-GHD adolescents with during transition (233) and young adults (238). However, it could be assumed that bone deficit in some patients of our cohort may be related, at least in part, to the fact that they have delayed or not yet attained their peak bone mass at the time of final height (386). Reviewing the literature, the precise age at which peak bone mass is achieved is still uncertain and varies by age, sex, evaluated sites and assessment methods. Boot et al estimated that the peak of bone mass in a healthy Caucasian population is attained between the age of 18-20 years in females and 20-23 years in males (387), however a Canadian study estimated timing of peak lumber spinal BMD occurs between 33-40 years in females and 19-33 years in males (388). It was generally believed that about 85–90% of final adult bone mass is acquired by the age of 18 years in females and 20 years in males (389). Late-onset puberty might be another contributing factors resulting in bone deficit in our cohort as late-onset puberty and timing of the adolescence growth spurt are inversely associated with peak bone mass in both genders as previously established (390-392).
Our study has also provided further evidence on gender differences and sexual dimorphism in the way of skeletal response to long-term rhGH replacement in CO-GHD. As previously reported (233,393,394), CO- GHD males unlike females had lower TB bone mineralisation for their size and height, which may reflect lower cortical bone density, whereas CO-GHD females appeared to have less mineralisation at the LS, which is a region of higher trabecular bone. It could be assumed that sexual differences in sites of bone deficit appears to be obvious contributors in sex differences in fractures risk distribution in males and females adult with CO-GHD reported previously (256).

Our analysis also showed that adolescents with congenital and IGHD had lower mineralisation in TB and LS than those with acquired and had MPHD. These findings clearly indicate that time of onset and aetiology of CO-GHD, but not additional pituitary hormone deficiencies may have a larger influence on accrual of bone mass in these patients, as those with acquired late onset GHD had a longer period of normal growth before the onset of GHD (395,396).
Given that, it seems the mild deficit in bone health in CO-GHD at final height still exists even over a prolonged period of childhood rhGH replacement therapy. Although several randomised control trials have shown that bone deficits in CO-GHD at final height were largely corrected with resuming rhGH therapy (397,398), it is still currently uncertain whether recommencement of rhGH

therapy in CO-GHD will result in normalization of detected deficit and reduced risk of fracture in adulthood (399).

To date, there have been relatively few studies comparing diagnostic accuracy of the different size adjustment techniques of DXA derivative bone and body composition parameters. In our results, all size adjustment techniques reduced the number of patients classified as abnormal (majority of SDS within the range of $\pm$ 2). In accordance with the Crabtree approach (381), the percentage of sarcopenic and osteopenic subjects was very small with no GHD patient in our study having a mixed muscle and bone defect. Currently, there is debate around the appropriate adjustment method to apply in assessment of bone in children with chronic conditions effecting their growth and maturation. Recently the international society for clinical densitometry (ISCD) recommended applying LS-BMAD and TB- BMC for height into routine clinical practice for investigating bone health in children and adolescents (83).

With regards to body composition in most studies after discontinuation of childhood rhGH treatment, a significant decrease is seen in LM, paralleled by an increase in FM in CO- GHD patients (233,236,245,246). In our study, no differences were found in LM between CO-GHD patients and healthy controls in either gender. However, when LM was adjusted for height, CO-GHD patients had a significant lower LM for height, but their TB-BMC were normally adapted for the reduction in LM. On the other hand, males with CO-GHD, but not females, had higher FM and FM for height SDS than control subjects. By contrast, higher A/G fat ratio, the measure of central adiposity, was in females with CO-GHD compared to matched controls.
In normally growing children, the relationship between LM and height is exponential, with the LM for height ratio increasing in males beyond age 20 years and in females until age 14 years, with no significant increases thereafter (400). In light of our data, it is unclear whether these gender differences are mediated by the pattern of response to rhGH replacement in our patients or by gonadal steroid hormones. Therefore, it was argued that continuation of rhGH after final height not only optimizes progression to peak bone mass, but also affects the late stage of gender-specific maturation to adult body composition (246).

Different authors speculate about possible factors being responsible for bone and body composition alteration in previously childhood rhGH treated subjects with CO-GHD. In our study, the values of bone or body composition were not correlated with clinical findings, stimulation GH peak/serum IGF-1 levels on re-testing at final height, or duration of childhood treatment as previous reported (224). However, FM increased with increasing numbers of other pituitary hormone deficiencies. In fact, due to the heterogeneity of our cohort, it was very difficult to examine the possibility of GHD as an independent factor in alteration of body composition in these subjects and to be certain to what extent other pituitary hormones, particular sex steroids contribute in these alterations.

The present study showed significant correlations between LM, but not FM, with BMC and BMD in both normal individuals and patients with CO-GHD, with regression analyses have confirmed LM to be the second strongest predictor of BMC in females alongside A/G ratio following bone area, after adjusting for age, sex and BMI. Increased LM perhaps results in more mechanical loading of body as compared to FM, resulting in a greater increase in bone mass (401). Given that, it seems vital that strategies are set onward to maximize the chances of attaining the highest possible peak bone mass through maximizing LM (e.g. through nutrition and increased weight bearing physical activity). Although FM has low influence on BMC/BMD in our study, fat distribution (android/ gynoid fat ratio) had also important factors, but only in females, in prediction of TB-BMC (402).

Study limitations

The present results should be interpreted within a number of potential limitations. In addition to small size cohort, cross sectional design and heterogeneity of our CO-GHD cohort did not allow us to establish any causal relationships between patient's bone deficit and GHD. Another potential limitation of our study is that we have derived predictive equations for calculating SDS of the ratio of LM, FM, and bone area for height from a fairly small local reference population. Furthermore, although we have used size correction of bone measures to avoid misinterpretation of two dimensional DXA, still this technique does not account for other important parameters of bone strength as micro architecture, intrinsic properties of materials that comprise bone (cortical geometry, trabecular, and material) and the three dimensional organization of the trabecular etc. We also have not accounted for physical activity as there was no available data.

4.7 Conclusion

In summary, adolescents with CO-GHD after final height showed a clear deficit in bone mass and density, with alteration in body composition compared to age/height matched healthy controls. Lower bone mass in our patients with congenital IGHD support the view that the onset and duration of GH deficiency per se could be responsible for part of the observed deficit. These findings may raise the question how GH deficiency may have resulted in deficient bone mass despite rhGH treatment. Further prospective longitudinal studies are needed to evaluate this finding in more depth.

CHAPTER 5

Bone Health and Body Composition in Childhood Onset Growth Hormone Deficiency at Time of Initial Evaluation and Retesting

5.1 Abstract

Background: Childhood onset growth hormone deficiency (CO-GHD) may cause some alterations in bone density and body composition. However, the direct mechanisms by which GHD effects bone health are not yet clearly defined.

Aims: 1- To evaluate musculoskeletal health and body composition in CO-GHD children and adolescents at the time of initial evaluation and retesting after final height.

2- To explore the relationship of bone mass and body composition parameters with bone metabolism and bone turnover biomarkers in subjects with CO-GHD.

Methods: This is a cross-sectional study of assessing bone health and body composition by imaging (DXA-pQCT) and biochemical assessment of patients with CO-GHD at the time of initial evaluation and retesting at final height.

Population: A total of 25 first time assessment group of children undergoing GH stimulation tests for investigation of short stature (naive GHD –15, median age (range) 10.9years (5.6, 16.0)), and 11 adolescents with CO-GHD undergoing biochemical re-evaluation at final height after withdrawal of rhGH therapy (persistent GHD-7, median age 16.7years (14.9, 18.6)) were involved in the study.

Results: After adjusting for age, height, and bone area, GH deficient individuals were not different in bone and body composition parameters as measured by DXA and pQCT from those who had normal GH levels at time of initial evaluation and retesting after final height. Assessing muscle strength by mechanograph, the median of maximum - force (F-max (kN) in naive GHD patients was significantly lower than normal subjects [0.5 (0.3, 2.8) vs. 2.7 (2.2, 3.3) respectively, p= 0.03] which was proportional to their tibia muscle cross sectional area. There were no significant differences in bone profiles and metabolism; (Calcium-Phosphate-Magnesium-Parathyroid hormone (PTH)-OH25-vit-D) and bone formation markers (bone-specific alkaline phosphatase - osteocalcin) among all studied groups. However, the bone resorption marker, C-terminal telopeptide (CTX), was significantly higher in naive GHD vs. normal of the first time assessment group (2.0 ng/ml (1.4, 3.9) vs. 1.6 ng/ml (0.9, 2.8), respectively, p=0.02). In univariate analysis, a significant and positive correlation was found between CTX and PTH levels at time of initial evaluation (r=0.46, p=0.02) and retesting (r=0.77, p=0.02) and CTX with duration of withdrawal rhGH to retesting (r= 0.73, p=0.04).

Conclusion: Subjects with CO-GHD appear to have normal bone mass and body composition at time of initial evaluation and retesting after withdrawal of rhGH at final height. However, significant lower muscle force and higher CTX was found in naive GHD compared to normal. Our results suggest that muscle force and serum PTH may be important determinants of bone health in subjects with CO-GHD. However, a large-scale study is required to verify our findings.

5.2 Introduction

Growth hormone (GH) is an important contributor for optimizing bone mass accrual and body composition during childhood and adolescence (403,404). The existing studies assessing the impact of childhood onset GH deficiency (CO-GHD) on bone and body composition thus far are not conclusive. Studies suggest that CO-GHD is associated with alterations in bone and body composition, resulting in developmental deficits in bone mass at time of diagnosis (88,89) and retesting at final height (263). However, bone mass data of children with CO-GHD has been difficult to disentangle because the majority of these data have been assessed using dual-energy x-ray absorptiometry (DXA), with few studies having used peripheral quantitative computed tomography (pQCT). Indeed, both DXA and pQCT methods can be challenging and provide misleading values when used in small and young children if no adjustment to variables of age, height and bone size are considered (83,405,406).

Despite the increasing number of studies investigating variables related to musculoskeletal development of CO-GHD, a direct mechanism underlying the effects of CO-GHD on bone mass and strength is not yet clearly defined. Considering the concept of a "Functional Muscle-Bone Unit" which has clearly reflected the relationship of muscle mass and force to bone mass and geometry in the developing skeleton during childhood and adolescence of normal population (68,407,408), it was suggested that GHD may initially produce a deficit in muscle mass and force that will ultimately affect bone geometry and density (92).

In addition to mechanical properties of bone mass, bone minerals and turnover are other factors involved in bone mass and strength in children and adolescents (60). Biochemical markers of bone turnover have been used in clinical practice to look at overall bone metabolism and dynamic bone turnover (409). It is well proven that GH plays a crucial role in regulating and maintaining the balance between bone formation and resorption (163). In parallel, studies have shown that bone formation and resorption markers were either low (410) or within normal reference ranges at baseline in individuals with CO-GHD prior to commencing recombinant human GH (rhGH) (235), or at time of retesting after withdrawal rhGH at final height (230).

In view of the above, our objective is, therefore, to study parameters indicative of bone density and bone structure in relation to body composition, muscle function, bone metabolism and biomarkers of patients with CO-GHD at time of initial evaluation prior to starting childhood rhGH treatment and at retesting after termination of childhood rhGH treatment at final height.

5.3 Subjects and Methods

5.3.1 Study design and subject

This is baseline data collected for the main longitudinal, controlled study of 6 months duration looking to evaluate the effect of weight bearing exercises-and rhGH replacement therapy on bone status and body composition in CO-GHD subjects at time of initial evaluation and retesting after terminating childhood rhGH therapy, detailed description of the study will be illustrated in chapter 8.

Inclusion criteria: 1- Children who are having GH stimulation tests as part of their assessment/investigation of short stature. 2- Adolescents with CO-GHD who attained final height and are having their GH axis re-assessed.

Exclusion criteria: 1- History of major (abdominal) surgery within the previous three months. 2- Skeletal abnormalities associated with joint and limb deformity. 3- Patient or family who in the investigators opinion are not able to comply with the trial protocol. 4- Children < 5 years of age.

The study protocol was approved by the national research ethics service and all participants and their care givers provided written informed consent.

5.3.2 Anthropometry measurements

Anthropometry measurements (weight and height) were measured at time of the scan and were converted to SDS scores using the British 1990 reference (384). Tanner staging was recorded from patients clinical cases note at time of clinical evaluation. Tanner stage I is defined as prepubertal, Tanner stages II and III are defined as early pubertal, Tanner stage IV is defined as late pubertal, and Tanner stage V is considered fully mature.

5.3.3 Biochemical assays

In 33/36 subjects, fasting blood samples were taken on the day of the stimulation tests (insulin tolerance test (ITT)/ arginine stimulation test as per local protocols of Scottish Paediatric Endocrine Group (SPEG) guidelines) after overnight fasting. Bone profiles and elements (Calcium, phosphate, magnesium, parathyroid hormone (PTH), 25-hydroxyvitamin D (25 (OH) D)) were measured immediately after blood sampling in Biochemistry laboratory in Royal Hospital for Children (RHC), Glasgow with standard methods and references ranges of CALIPER (411). The remainder of the samples was separated immediately by centrifugation for 5min at 2500 rpm and then stored at -80◦C until the assays were performed. Serum Osteocalcin (OC) was measured by enzyme immunoassay (EIA) using kit manufactured by BioSource, Nivelles, Belgium with intra- assay coefficients of variation (CV) 0.8% –3.1%. Serum bone-specific alkaline phosphatase (BAP) was measured by Ostase® BAP immunoenzymetric assay (Immunodiagnostic Systems Ltd (IDS Ltd, Boldon, UK) with an intra-assay CV of 0% to 2.3%. Serum cross linked C-telopeptide of type I

collagen (CTX) was determined using serum crossLaps® ELISA (IDS Ltd, Boldon, UK) with an intra-assay CV of 1.6% to 2.0%. The ELISAs were performed by Martin McMillan in the department of child health, RHC, Glasgow.

5.3.4 DXA parameters

DXA scans were performed at total body (TB), lumbar spinal (LS) in 32/36 of the study participants using a narrow fan beam lunar prodigy densitometer (GE Medical Systems, Waukesha, Wisconsin, U.S.A) using the Encore software (Version 8.80.001). To minimise the size effects in DXA bone densitometry, bone and body composition parameters were corrected for bone area/height/age as described in chapter-4. In this chapter, measurement of TB/LS BMD Z scores $_{\text{height age}}$ and BMC for bone area SDS were excluded in children aged <6 years.
All DXA scans were carried out by Dr Sheila Shepherd at RHC, Glasgow.

5.3.5 Peripheral quantitative computed tomography (pQCT)

In this study, 28/36 subjects had Tibia pQCT using (XCT-2000; Stratech, Pforzheim, Germany software v5.5). All pQCT scans were carried out by Dr Sheila Shepherd at (RHC), Glasgow. Tibia length was measured manually from the medial malleolus to the superior margin of the medial condyle. The average of two measurements was used to determined tibia length. The 66% measurement site was calculated (tibia length [cm] × 0.66), measured distally from the medial malleolus and marked on the subject's calf with non-permanent ink. The subject's leg was then extended into the instrument gantry (Figure 5-1, A). A scout scan was performed to visualize the distal growth plate and reference line placed at the most proximal line of the growth plate or at the end plate in case of the fussed growth plate (Figure 5-1, B). After establishing the distal line, the 4% distal cross section was identified and measured by scanner. Total volumetric bone mineral density (TvBMD) and trabecular density (vBMD) were determined at the 4% site, but cortical density (vBMD) measured at the mid-shaft (38% tibia). Tibia geometry: cortical thickness (mm), endosteal and periosteal circumferences were measured at the 38% site. Muscle and fat mass were determined at the 66% site, (Figure 5-1, C, D). The pQCT bone outcomes were converted to Z-scores relative to age, sex and height based on recent references data (412). We calculated age and height Z scores for tibia bone geometries at only 38% site. Age Z scores were calculated for (CvBMD at 38%) and (TrvBMD at 4%).

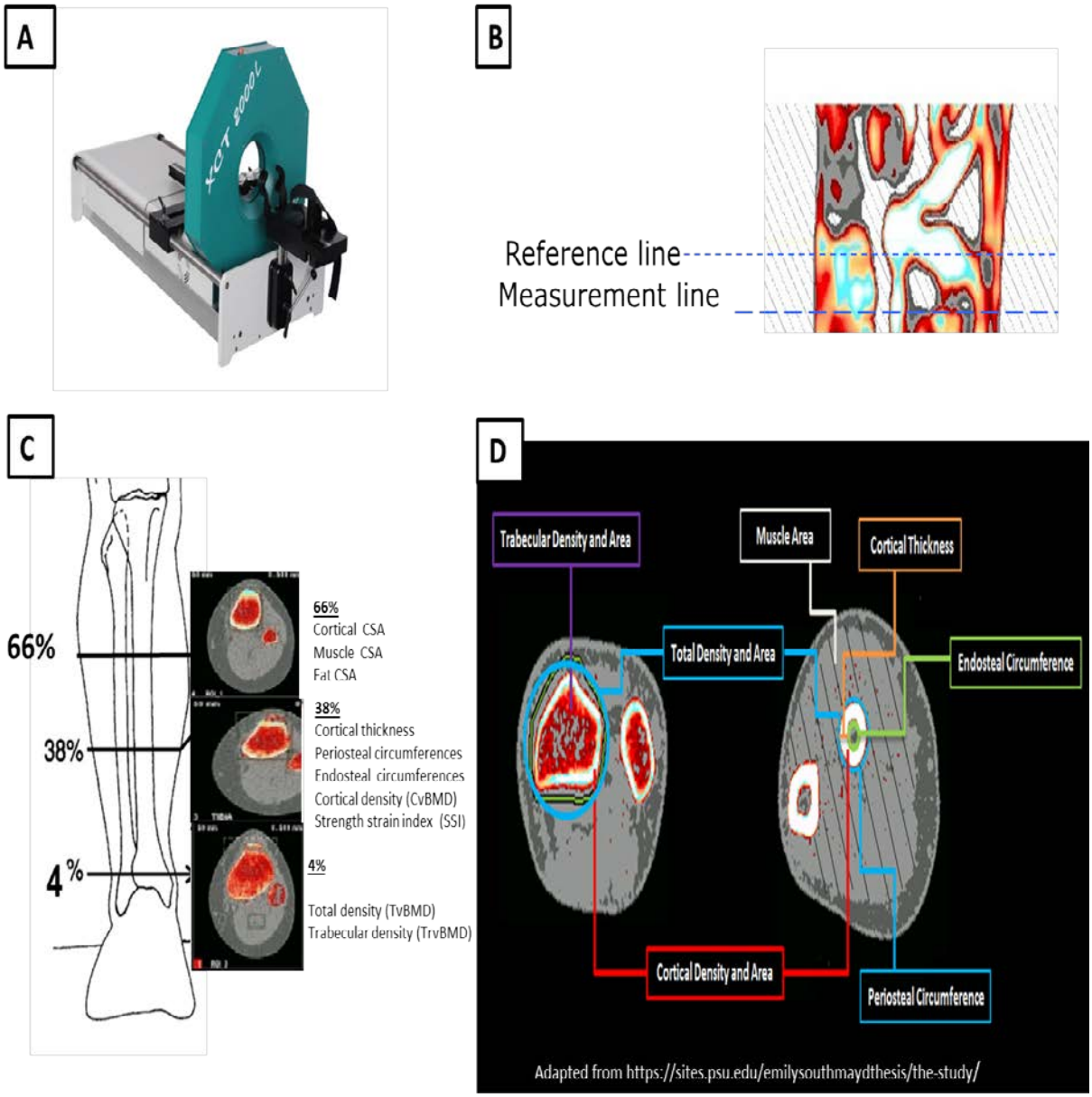


Figure 5-1 Tibia pQCT.

A-Tibia pQCT(Adapted from www.galileo-training.com)

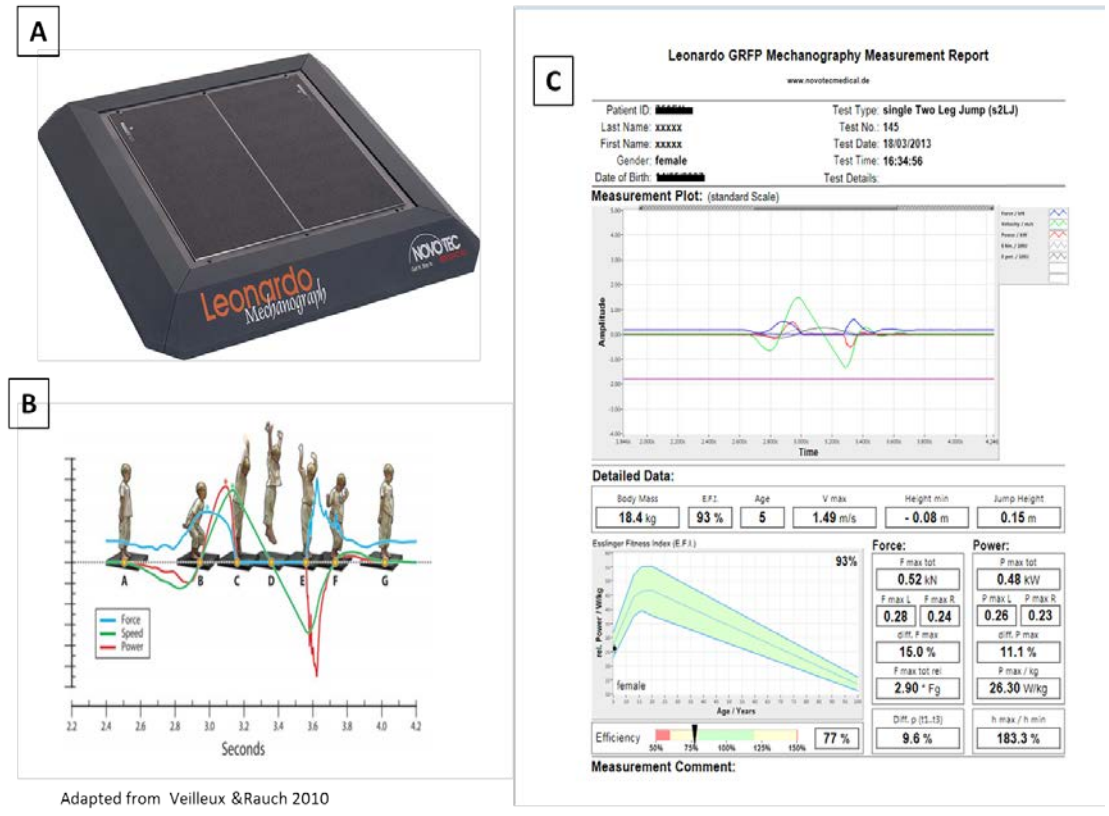
B-Determine reference line before scanning

C-Scan sites, slides and outcomes of tibia pQCT: 4%, 38% and 66%

D-Tibia PQCT outcome as measured at each site (Adapted from https://sites.psu.edu/emilysouthmaydthesis/the-study with permission)

5.3.6 Muscle strength

The Leonardo mechanograph (version 4.2-Novotecc Medical GmbH, Pforzheim, Germany) was used to measure lower limb muscle force, power, velocity, jumping height and efficiency of the movement. There are five mechanographic tests; procedures include multiple one-legged hopping, multiple two-legged hopping, single two-legged jump, heel-rise test, chair-rise test, but single two-legged jump is the commonly used in children to evaluate the maximal force to which the tibia is exposed(76). A single two-legged jump with bare feet (wearing only socks) was assessed as a counter movement with freely moving arms. The individual stood on the plate force, and each foot was placed on one section of the jumping force plate. Each participant was instructed to jump as high as possible for at least three times and the result of highest jump was included. Parameters used for analysis were jump height (m), maximum-velocity (V-max (m/s), Esslinger fitness index (EFI (%), maximum - force (F-max (kN), maximum-power (P-max (kW), efficiency (%) of the movement, (Figure 5-2).



Adapted from Veilleux &Rauch 2010

Figure 5-2 Leonardo mechanography.

A - Leonardo mechanography software version 4.2 (used in this thesis). Force plate composed of two symmetrical force plates that separate the platform into a left and a right, with eight sensors each sensor recording force the vertical ground reaction force exerted on the platform at a sampling frequency of 800 Hz.

B- Single two-legged jump. The Force-Time, SpeedTime and Power-Time curves are shown as well as the phases of the movement corresponding to the indicated points on the Speed-Time curve (adapted from Veilleux &Rauch (76) with permission).

C- Mechanography printed measurement report

5.4 Statistical Analysis

Data were reported as the median and range (minimum-maximum). Categorical variables were compared using Fisher's exact and chi-square tests, and continuous variables using the Mann–Whitney test. Correlations among variables were determined by using the Spearman's test. All analysis performed using the Minitab17 software (Minitab, Coventry, UK), with significance set at a level of 5% ($P<0.05$). All graphs were performed using GraphPad Prism 6 software (GraphPad Software, San Diego, CA, USA).

5.5 Results

5.5.1 Patient characteristics

63 eligible children were approached children during the period August 2012 to November 2013 from the single tertiary centre (RHC). A total of 36 children were recruited, and 27 were excluded for a range of reasons as seen in Figure 5-3.

36 children who enrolled in the study were categorised according to their timing of assessment into: 25/36 subjects who were undergoing GH stimulation tests for short stature (first time assessment group) and 11/36 subject with CO-GHD who were undergoing retesting after withdrawal rhGH therapy at final height (retesting group).

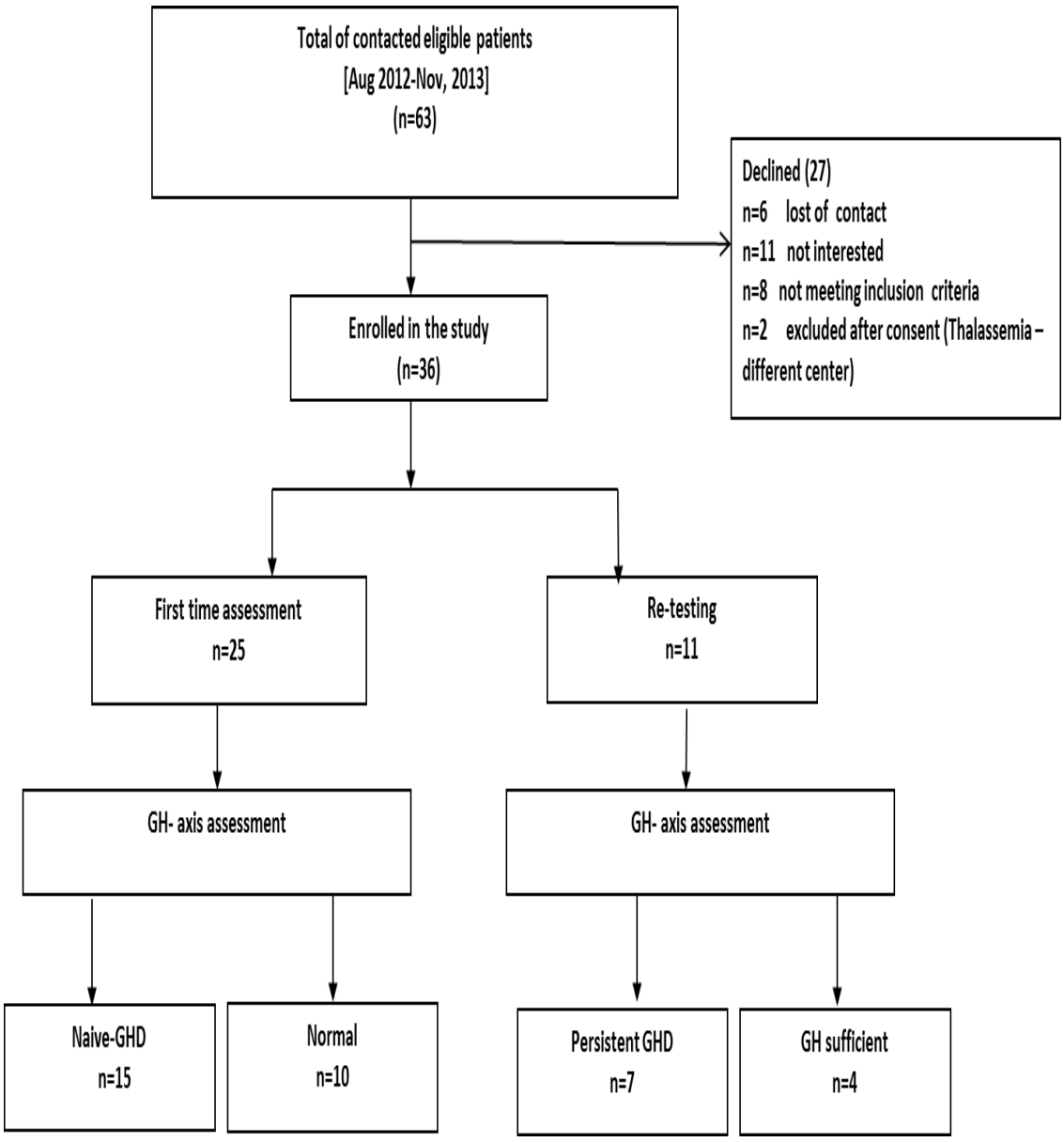


Figure 5-3 Flow diagram of study recruitment

5.5.2 First time assessment group

In all children GH levels were measured either after one (n=21) or two (n=4) GH stimulation testes (ITT, arginine stimulation test). GHD was defined as low GH peak response on stimulation test below (6.6µg/l).
A total of 15 out of 25 were confirmed as having naive GHD. All the 15 naive GHD have isolated GHD. Pituitary MRI was performed in all of the 15 naive GHD, seven patients showed structural hypothalamic-pituitary abnormalities (n=2 ectopic pituitary, n= 2 arachnoid cyst, nerve gliomas, craniopharyngioma, empty sella syndrome). Six patients showed normal MRI (idiopathic GHD), and no access to MRI reports in two patients. Among those who were GHD, five children had another known illness (21-trisomny)-(neurofibromatosis and precocious puberty) - (two with obesity (BMI >+2 SDS)) – (mosaic variegated aneuploidy).
10 of the 25 had normal GH levels (normal) on stimulation testes, of those, 6/10 were "short normal" children with no known other illness, and n=4 have other conditions: (Autoimmune polyendocrinopathy ectodermal dystrophy), (Mitochondrial disease with primary ovarian failure), (Juvenile rheumatoid arthritis), and (45/46 XY gonadal dysgenesis).

5.5.3 Retesting group

A total of n=11 adolescents with CO-GHD were first diagnosed at age 9.5yrs (2.6, 11.4) and rhGH treated during childhood at age 10.8 yrs (7.1, 13.6) with median duration of treatment is 4.3 yrs(2.9,7.8). Five subjects of the 11 had isolated GHD, four of them had idiopathic GHD, and one had ectopic posterior pituitary on MRI scan. Of the remaining six of the 11, they had multi pituitary hormones deficiencies (MPHD) and received hormonal replacement therapy, where necessary, with glucocorticoid, Levothyroxine, sex steroid (Estradiol - Testeterone) and desmopressin. Of those, one had hypoplastic pituitary on MRI scan and five had tumour related cranial irradiation. All patients' ceased GH treatment at final height to re-test their GH status at age 15.7 yrs (14.7,17.9) after a period of 0.6 yrs (0.2, 1.0) off rhGH either by stimulation tests (n=9) and /or IGF-1 levels alone(n=3). After re-testing, 7/11 of them were reconfirmed to have persistent GHD (low GH peak <5, and or low IGF1 <-2 SD for age references) and were eligible for adult GH replacement therapy, the other 4 were no longer GHD after retesting (GH-sufficient).

5.5.4 Anthropometric and biochemical characteristics

A summary of the baseline characteristics of the first time assessment and the retesting groups are given in Table 5-1.

The anthropometric characteristics were similar in those with naive GHD and normal of the first time assessment group and that was even after excluding subjects with known co-morbidities from both groups. There were also no significant differences in age, weight or BMI between the group of previously treated CO-GH deficient-patients who had persistent GHD and those with GH sufficient.

Regarding the puberty status of the first time assessment group, 10/15 (66%) of naive GHD and 5/10 (50%) of normal were prepubertal (tanner stage I). Of remaining, 3/15 (20%) of naive GHD and 2/10 (20%) normal were tanner stage II, and 2/15 (13%) naive GHD, 2/10 (20%) normal were stage III, with only one normal subject was between stage III-IV.

All retested adolescents with CO-GHD were considered fully mature- tanner stage V.

Table 5-1 Auxological and clinical characteristics of the first time assessment and the retesting groups

	First time assessment (n=15)			Retesting (n=11)		
	Naive GHD (n=15)	**Normal (n=10)**	**P-value**	**Persistent GHD (n=7)**	**GH sufficient (n=4)**	**P-value**
M/F	13/2	7/3	0.30	3/4	1/3	0.50
Age(yrs)	10.9 (5.6, 15.2)	12.1 (5.8, 16.5)	0.90	16.6 (14.9, 18.6)	16.8(16.3, 20.4)	0.73
Anthropometry						
Height (cm)	129.0 (97.7, 152.2)	130.1(96.1, 153.3)	0.76	158.7(152.7, 179)	153.5(145.3, 166.4)	0.35
Height -SDS	-2.5 (-3.4, 1.3)	-2.2 (-4.6, -0.1)	0.51	-1.2 (-1.9, 1.2)	-1.6(-3.0, 0.5)	0.50
Weight (kg)	26.5 (15.0, 71.4)	29.3 (13.3, 56.7)	0.94	60.6(45.6, 71.2)	56.0(37.6- 66.7)	0.63
Weight-SDS	-1.8 (-3.6, 1.9)	-1.3 (-4.7, 0.7)	0.93	0.6 (-1.8, 1.4)	0.0(-3.2- 1.1)	0.63
BMI	16.5 (14.2, 32.3)	17.2(14.4, 24.7)	0.97	22.4(18.8, 28.0)	23.8(17.8-24.1)	0.65
BMI-SDS	0.0(-1.8- 3.0)	0.0 (-2.4, 1.6)	0.57	0.9 (-1.1, 2.0)	1.0(-1.4- 1.1)	0.59
GH-peak(µg/l)	2.6 (0.7, 4.7)	8.0 (6.7, 22.3)	**<0.01**	2.0 (0.1, 3.8)	8.3(6.4, 10.2)	0.05
IGF1 levels(ng/ml)	65.0 (14.0, 433.0)	85.5(28.0, 295.0)	0.52	141.0 (18.0, 294.0)	241.5(117, 327.0)	0.24
IGF1 levels SDS	-3.2 (<-5.0, 0.3)	-2.0 (-4.5, -0.9)	0.72	-3.2 (<-5.0, -1.3)	-2.0 (-3.5, -0.9)	0.27
Tanner stages n (%)						
I	10 (66%)	5(50%)	0.44			
II	3 (20%)	2(20%)	0.95	-	-	
III	2 (13%)	2(20%)	0.94			
IV	0	1(10%)	0.20			
V	0	0	-			
Retesting data						
Age of childhood diagnosis (yr)				9.5 (2.6, 10.3)	11.4(7.0, 12.0)	0.23
Age of start treatment(yr)				10.3 (7.1, 13.6)	11.4(7.0, 12.0)	0.76
Duration of childhood rhGH (yr)				4.7 (2.9, 7.8)	8.0 (4.3, 10.2)	0.24
Age of stopping rhGH (yr)				15.9 (14.4, 17.9)	17.0(15.7, 20.0)	0.36
Duration of stopping rhGH (yr)				0.6(0.2, 1.0)	0.7(0.4, 1.0)	0.51

5.5.5 Bone density and body composition (DXA)

There were no differences in TB-BMD between the naive GHD and those with GH normal of the first time assessment group with none of subjects having a BMD height age Z score below -2. The median of TB-bone mineral content (BMC) tended to be lower in naive GHD but not significant (1069.6(g) (432.5, 2169.7) vs.1325.9 (g) (34.0, 1797.1) respectively, p=0.76) and all were within (± 2 SDS) when adjusted for bone area, height and LM, Figure 5-4.

There were also no differences in LS-BMD and BMC between naive GHD and normal of the first time assessment group, and the same pattern was observed after the estimation of volumetric LS-BMAD and BMAD SDS as seen in Table 5-2, Figure 5-5. Two of the naive GHD had LS-BMAD SDS below -2 SDS (a girl with idiopathic GHD and down syndrome- a boy with ectopic posterior hypoplasia pituitary).

Body composition compartments (LM-FM) were also similar across groups of the first time assessment, Figure 5-6. Two naive GHD had their FM for height SDS >+2 SDS (a boy with idiopathic GHD- boy with ectopic posterior pituitary).

Similar to the first time assessment group, the retesting group of adolescents who were previously treated with childhood rhGH exhibited areal and volumetric bone density and body composition parameters values similar to those who were GH sufficient after retesting at final height even after adjustments for height, bone area, and LM, as seen in Table 5-2 and illustrated in Figures 5-4, 5-5, 5-6. Only one girl with persistence GHD (idiopathic-isolated) had here FM for height SDS > +2.

Table 5-2 Bone parameters and body composition as measured by DXA in the first time assessment and the retesting groups

	First time assessment (n=23)			Retesting (n=10)		
	Naive GHD (n=14)	**Normal (n=9)**	**p-value**	**Persistent GHD (n=7)**	**GH sufficient (n=3)**	**p-value**
TB-BMD (g/cm^2)	0.9 (0.7, 1.1)	0.9 (0.7, 1.1)	0.99	1.0 (1.0, 1.2)	1.0(0.8, 1.1)	0.83
TB-BMD-Z-score-$_{age}$	-0.5 (-1.9, 1.4)	-1.5 (-2.3, 0.1)	0.07	-0.9(-2.2, 0.9)	-1.4(-4.1, 0.6)	0.59
TB-BMD Z score-$_{height\text{-}age}$	0.7 (-0.2, 3.6)	0.5 (-1.0, 2.2)	0.53	-0.1(-1.5, 2.1)	-0.6(-1.3, 0.3)	0.67
TB-BMC(g)	1069.6 (432.5, 2169.7)	1325.9(34.0, 1797.1)	0.76	1992.3 (1721.9, 3051.0)	1851.9(1074.0, 2162.8)	0.39
TB-BMC for BA SDS	0.4 (-0.3, 2.1)	0.4 (-0.7, 2.2)	0.85	-0.2(-0.5, 0.6)	-0.1(-0.6, 0.1)	0.09
TB-BMC for LM centile	66.0 (21.0, 94.0)	47.0 (9.0, 97.0)	0.86	74.0 (9.0, 97.0	26.0(25.0, 68.0)	0.39
TB-BA(cm^2)	119.0 (594.0, 1959.0)	1367.0 (521.0, 1736.0)	0.89	19882.0 (1714.0, 2481.0)	1826.0(1301.0, 2001.0)	0.34
TB-BA for height-SDS	-0.7 (-2.4, 4.6)	-0.1 (-3.4, 1.0)	0.90	-0.1 (-0.5, 3.2)	-1.2(-1.4, 0.8)	0.13
LS-BMD(g/cm^2)	0.7(0.4, 1.0)	0.8 (0.5, 1.0)	0.45	1.1(0.9, 1.2)	1.0(0.7, 1.1)	0.54
LS-BMD-Z $_{age}$	-1.2 (-2.8, 1.1)	-1.4 (-2.3, -0.5)	0.76	-0.4 (-2.3, 0.7)	-1.6(-3.8, -0.7)	0.45
LS-BMD-Z $_{height\text{-}age}$	0.2 (-2.4, 1.3)	-0.1 (-1.2, 1.6)	0.90	0.8 (-1.1, 1.3)	-0.6(-1.4, 0.3)	0.28
LS-BMC(g)	17.3(6.8, 31.0)	22.0(6.4, 32.5)	0.45	40.1(28.1, 57.3)	35.6(19.7, 44.7)	0.67
LS-BMC/BA-SDS	0.0 (-0.9, 1.9)	-0.1(-0.5, 2.6)	0.54	0.3(-0.7, 1.2)	-0.3(-0.9, -0.1)	0.29
LS-BMAD(g/cm^3)	0.28 (0.19, 0.37)	0.24 (0.22, 0.29)	0.96	0.35 (0.30, 0.35)	0.36 (0.31, 0.37)	0.39
LS-BMAD-SDS	0.2(-2.5, 2.3)	-1.4 (-3.2, -0.2)	0.28	-0.2 (-1.7, 1.0)	-0.7(-1.7, -0.5)	0.53
LM(kg)	22.8(11.4, 36.9)	23.0(10.3, 37.4)	0.87	37.8(28.3, 50.2)	30.9(22.8, 38.9)	0.38
LM for height centile	35.0 (19.0, 95.0)	43.0 (2.0-88.0	0.87	66.0 (21.0, 93.0)	45.0(5.0, 49.0)	0.59
FM(kg)	4.9(1.5, 31.8)	4.1(1.9, 15.1)	0.35	17.3(21.1, 28.1)	18.0 (12.7, 24.7)	0.99
FM for height-SDS	0.0 (-1.7, 4.2)	-0.3 (-1.4, 0.9)	0.19	1.0(0.4, 2.8)	0.6 (0.1, 1.0)	0.20
A/G ratio	0.7(0.5, 1.2)	0.6(0.4, 1.1)	0.24	1.1(0.7, 1.2)	0.8 (0.8, 1.0)	0.24

TB: total body; LS: lumbar spine; BMD: bone mineral density; BMC: bone mineral content; BMAD: bone mineral apparent density; BA: bone area LM: lean mass; FM: fat mass; A/G: Android/Gynoid fat ratio.

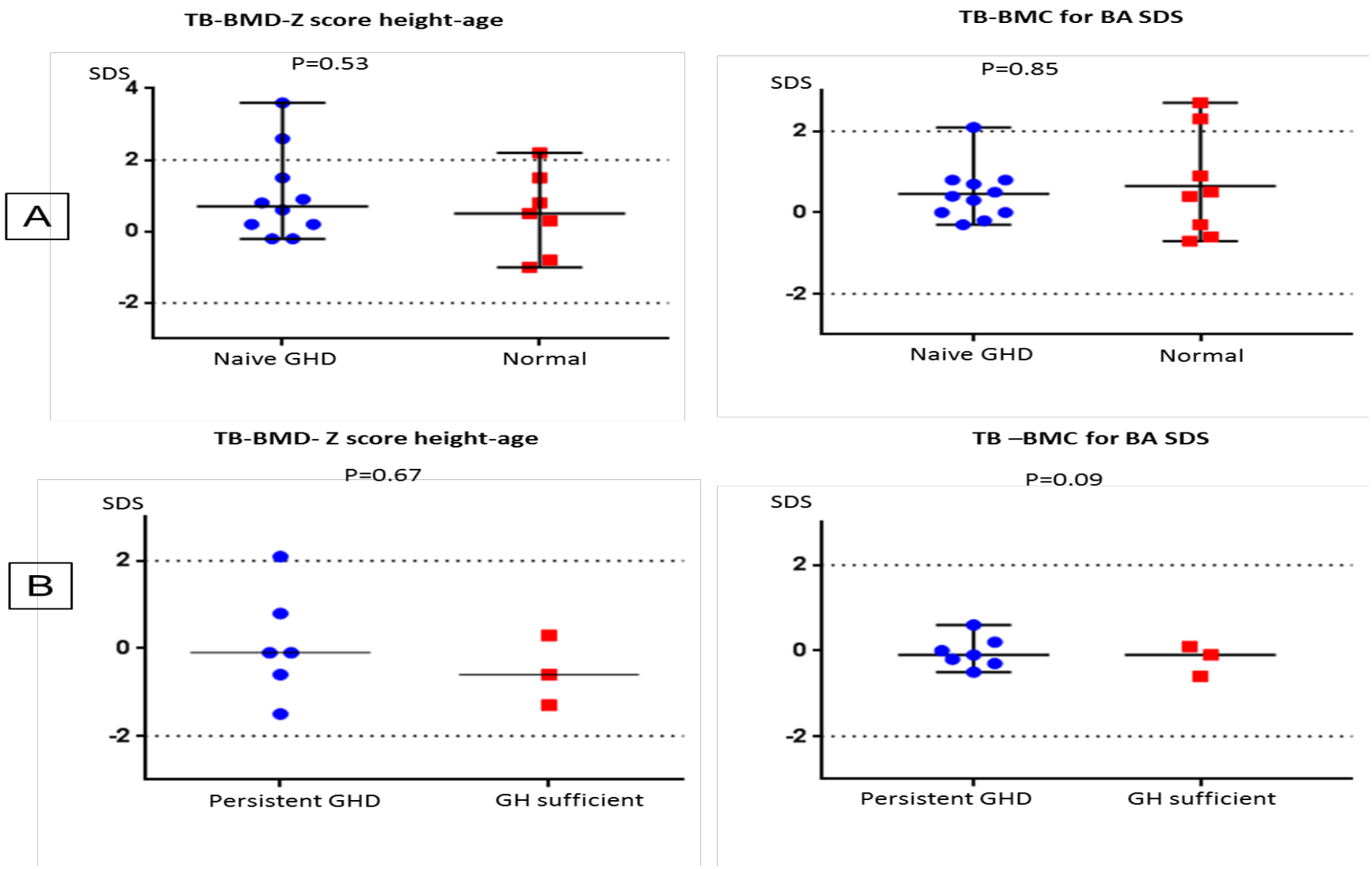


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Figure 5-4 Total body bone density and mineralisation of the first time assessment (A) and the retesting (B) groups.
TB, total body; BMD: bone mineral density; BMC: bone mineral content; BA, bone area

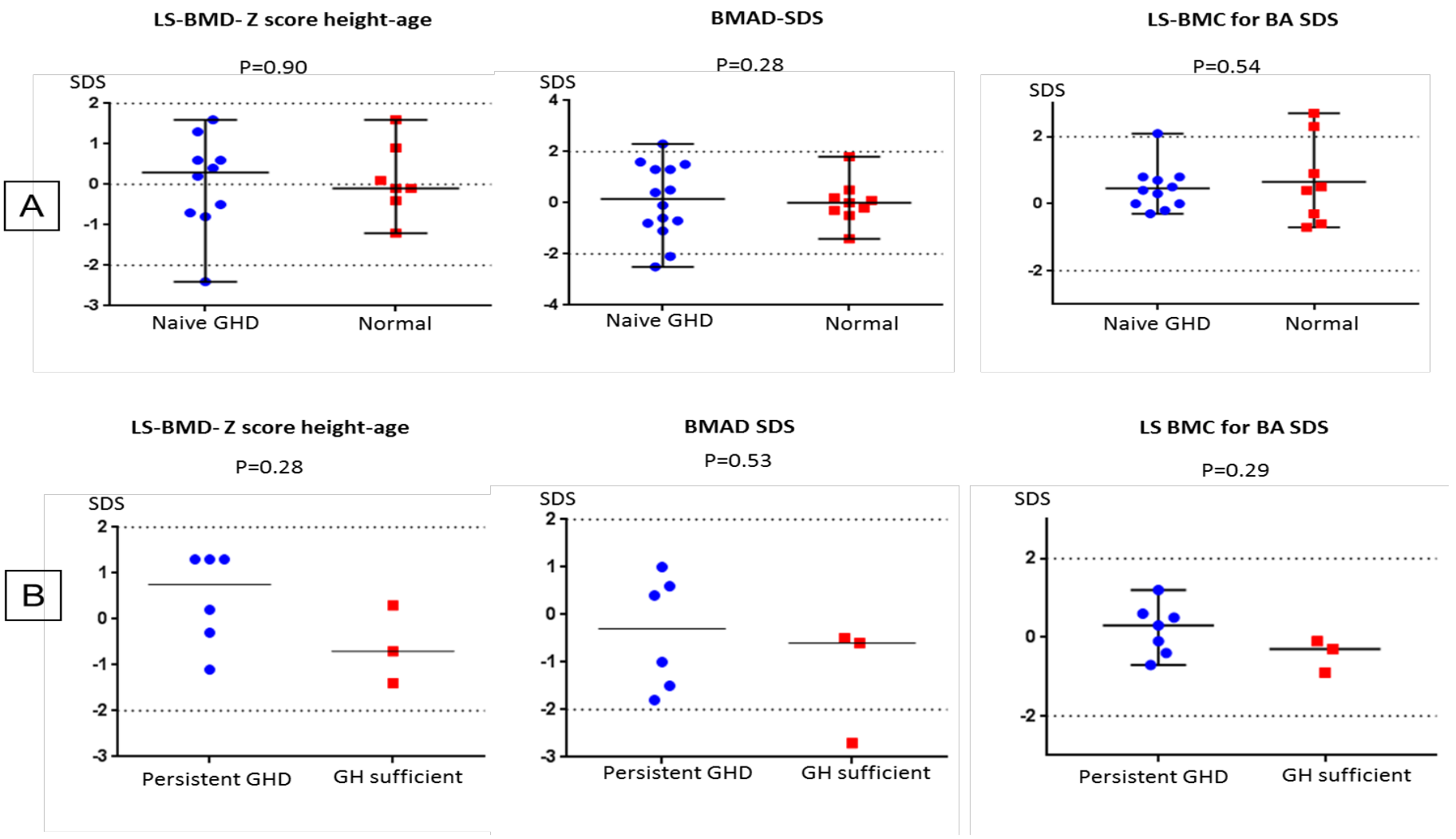

Figure 5-5 Lumber spine bone density and mineralisation of the first time assessment (A) and the retesting (B) groups.
Individual values (median-range) (blue circle, CO-GHD; Red Square, normal).

TB, total body; BMD: bone mineral density; BMC: bone mineral content; BMC/BA SDS

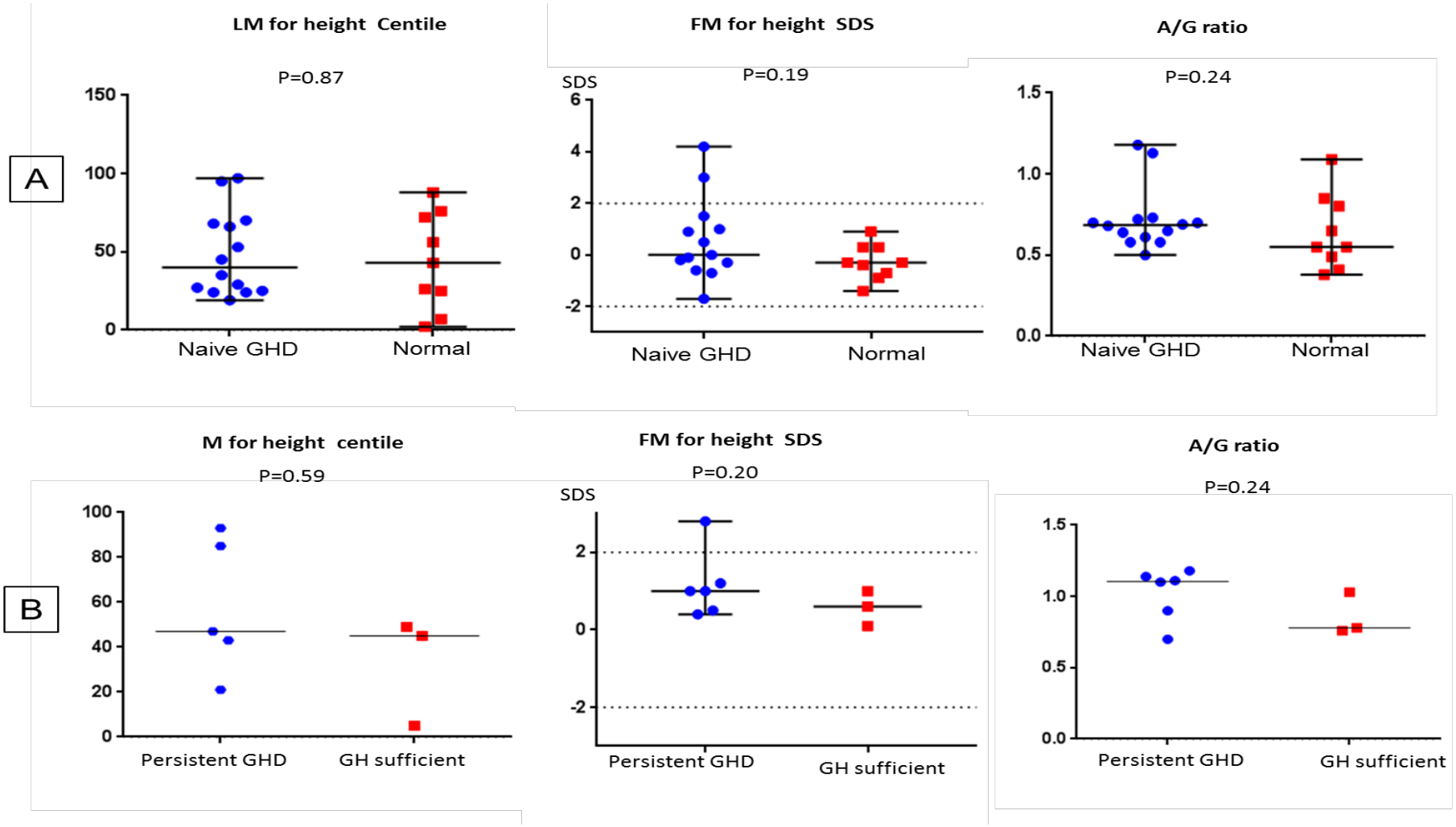

Figure 5-6Body composition parameters of the first time assessment (A) and the retesting (B) groups.
Individual values (median-range) (blue circle, CO-GHD; Red Square, normal). LM: lean mass; FM: fat mass; A/G: Android/Gynoid fat ratio

5.5.6 Bone geometry and density (pQCT)

Table 5-3 presents tibia pQCT parameters at each site of each group.

The absolute values of bone geometries and height z scores were not different between the GHD and normal in the first time assessment, Figure 5-7. The median values of total density, cortical density and trabecular density and age-Z scores were also similar naive GHD vs. normal, Figure 5-8.

In the retesting group, significantly wider periosteal circumferences at the 38% site in those with persistence GHD compared to GH sufficient (74.0 mm (65.7,77.5) vs. 60.0 mm (57.5, 64.2) respectively, p=0.02) and slightly more wider endo-oesteal circumferences 47.3mm(37.6, 53.3) vs. 37.3mm (33.3, 42.8) respectively, p=0.09) without any differences in medians of cortical thickness among the groups. However, these observations were not evident when adjusted for height Z scores, Figure 5-7.

Cortical density (CvBMD) in those with persistent GHD was significantly lower than those who were GH sufficient (1155.9 mg/cm^3 (1123.6, 1162.4) vs. 1171.0 mg/cm^3 (1165.8, 1177.1) respectively, p=0.02) but not when adjusted for age Z score (0.8 (-0.2, 2.2) vs. 0.7 (0.6, 1.1), p=0.90), with no significant differences in total and trabecular density across the groups, Figure 5-8.

No other tibia pQCT bone and composition parameters showed any differences between those with persistent GHD and GH sufficient in the retesting group, Figure 5-9.

Table 5-3 Tibia bone geometry and density as measured by pQCT in the first time assessment and the retesting groups

	First time assessment (n=19)			Retesting (n=9)		
	Naive GHD (n=13)	**Normal (n=6)**	**P value**	**Persistent GHD (n=6)**	**GH sufficient (n=3)**	**P value**
Cortical thickness(CTh) 38%(mm) CTh height- z-score	3.3 (1.6, 4.2) 0.4 (-1.4, 3.6)	3.0(2.4, 4.9) 0.6(-1.9, 1.8)	0.95 0.95	3.9(3.3, 5.9) -0.4(-1.4, 1.0)	3.9(2.7, 4.3) -1.0(-2.7, 1.3)	0.89 0.69
Periosteal circumference (PC) 38%(mm) PC height-z-score	58.3 (43.1, 78.8) 0.2 (-0.6, 3.8)	58.7(47.4, 72.6) 0.0(-1.7, 1.6)	0.95 0.56	74.0(65.7, 77.5) -0.1(0.8, 2.3)	60.0(57.5, 64.2) -1.5(-3.2 , 0.3)	**0.02** 0.24
Endosteal circumference (EC) 38% (mm) EC height-z-score	38.4 (31.7, 60.2) 0.1 (-1.6, 3.5)	35.4(31.2, 51.6) -0.2(-1.4, 1.9)	0.49 0.56	47.3(37.6, 53.3) 0.6(-1.8, 1.9)	37.3(33.3, 42.8) -0.6(-2.2, 0.3)	0.09 0.24
Total density(TvBMD) 4%(mg/cm^3)	301.7(213.9, 720.2)	306.3(238.7, 347.8)	0.71	279.8(212.4, 351.0)	308.7(170.1, 311.5)	0.89
Trabecular density (TrvBMD) (mg/cm^3) 4% TrvBMD-age-z-score	204.8 (144.2, 281.3) -0.3 (-0.2, 2.0)	204.1(172.3, 313.2) 0.2(-1.4, 1.8)	0.63 0.62	220.7(161.0, 268.5) -0.4(-2.6, 1.0)	193.8(117.8, 252.1) -1.0(-4.3, 0.7)	0.51 0.51
Cortical density (CvBMD) 38% (mg/cm^3) CvBMD –age-z-score	1067.2(999.9, 1115.9) 0.2(-0.8, 2.1)	1077.6(1039.2, 1168.0) 0.6(-2.0, 2.6)	0.54 0.89	1155.9 (1123.6, 1162.4) 0.8(-0.2, 2.2)	1171.0(1165.8, 1177.1) 0.7 (0.6, 1.1)	**0.02** 0.90
Strength strain index (SSI) 38%	696.4(217.6, 1267.1)	781.1(305.1, 1067.7)	0.76	1227.1(1078.6, 1558.0)	832.3(808.5, 1193.7)	0.15
Cortical CSA 66% (mm^2)	191.1 (77.2, 317.5)	217.2(71.2, 280.5)	0.98	295.6(266.0, 445.2)	278.5(165.7, 304.7)	0.51
Muscle CSA 66% (mm^2)	3488.0(77.8, 6551.3)	3567.3(368.5, 4633.2)	0.90	5163.8(4003.7, 8176.2)	3996.5(3356.2, 5247.2)	0.29
Fat CSA 66% (mm^2)	1618.0 (789.5, 5311.0)	1411.8 (1118.0, 2964.7)	0.63	2462.2(1269.2, 3543.0)	2817.2(2358.5, 3247.7)	0.58
Bone/Muscle ratio	5.5 (3.8, 6.6)	5.2 (3.0, 7.9)	0.84	5.5 (4.8, 6.6)	5.8 (4.9, 6.9)	0.69

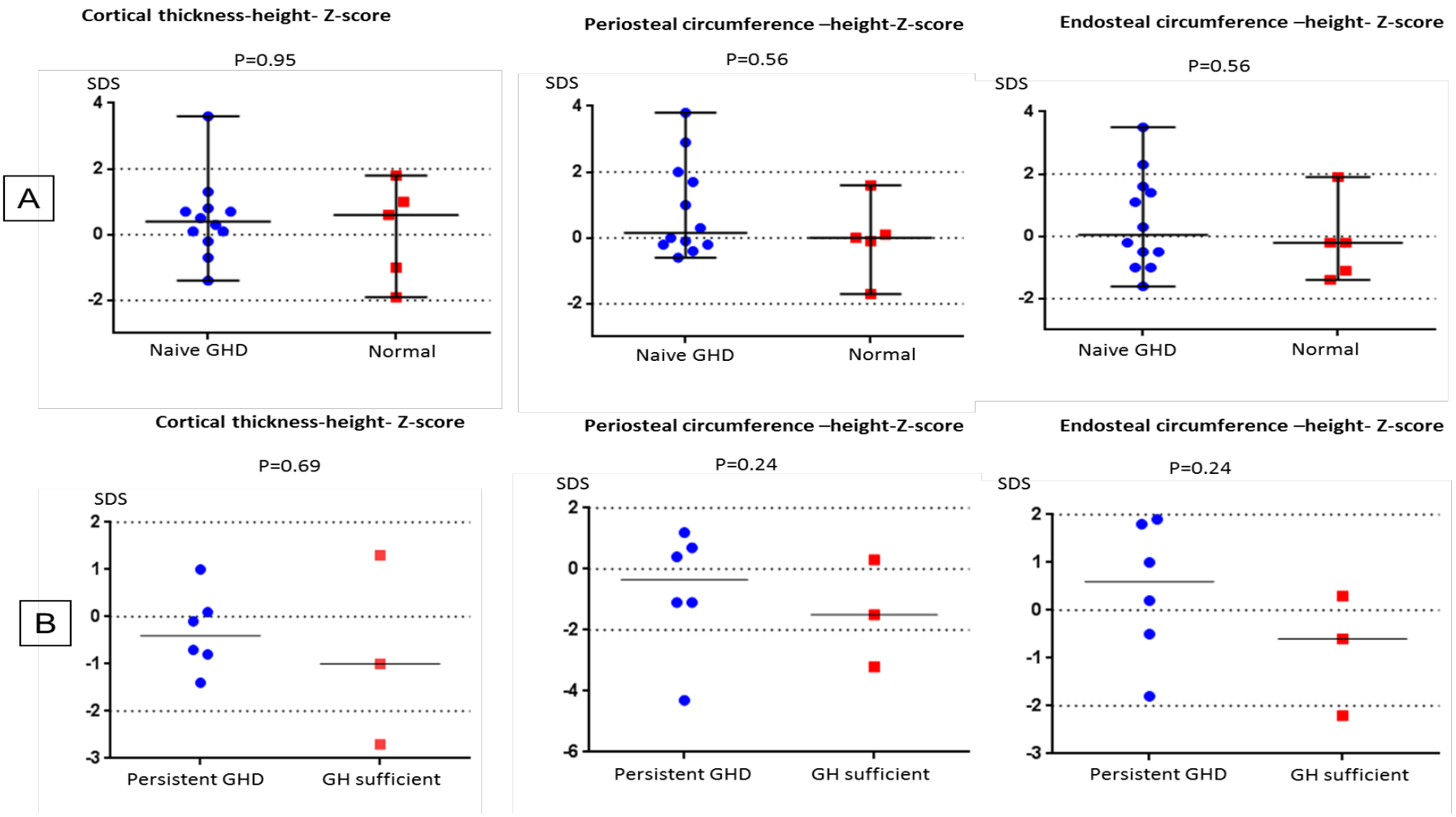

Figure 5-7 Tibia bone geometry at 38% site of the first time assessment (A) and the retesting (B) groups.
Individual values with median (range) (blue circle, CO-GHD; Red Square, normal).

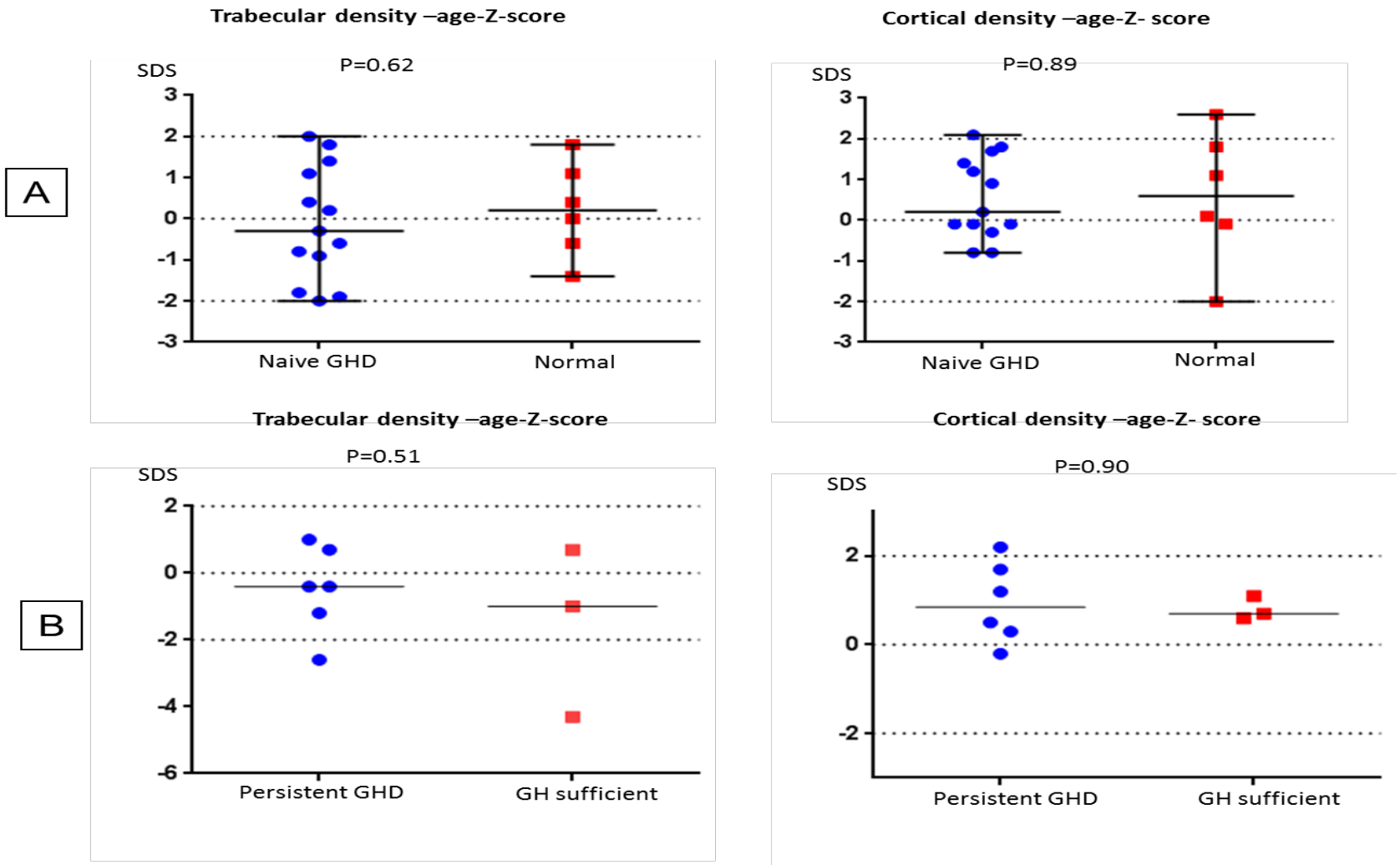

Figure 5-8 Tibia PQCT measurement of trabecular density, and cortical density in the first time assessment (A) and the retesting (B) groups.

Individual values with median (range) (blue circle, CO-GHD; Red Square, normal).

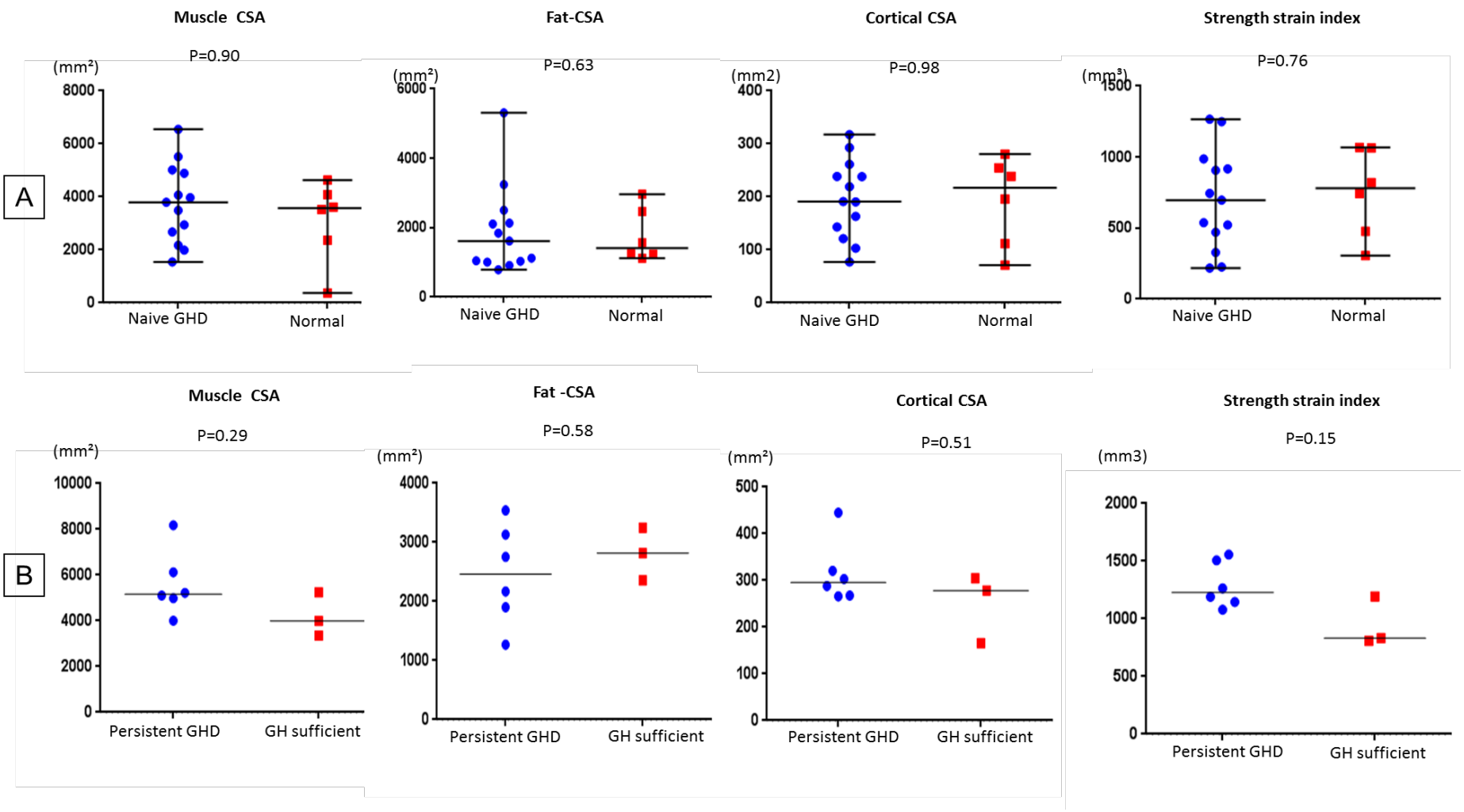

Figure 5-9 Tibia pQCT measurements of cortical bone, muscle and fat cross sectional area of the first time assessment (A) and the retesting (B) groups.

Individual values with median (range) (blue circle, CO-GHD; Red Square, normal).

Mus-CSA: Muscle cross-sectional area; Fat-CSA: fat cross-sectional area; cortical CSA: cortical cross-sectional area; SSI: Stress-strain index

5.5.7 Bone mineralisation and biomarkers

There were no significant differences in serum calcium, phosphorus, and magnesium between GHD and normal among the first time assessment and retesting groups and the majority were within normal range, Table 5-4, and Figure 5-10.

The majority of the first time assessment group had inadequate vitamin D levels (< 75 nmol/l). 25(OH) D levels tended to be lower in normal subjects vs. naive GHD within the first time assessment group, although not significantly (55.7 (23.0, 102.0) naive GHD vs. 35.5 (20.0, 69.0) normal, P=0.16). The percentages of 25(OH) D deficiency (<50 nmol/l) in the whole cohort of the first time assessment was high with 5/15(34%) of the naive GHD: 6/10 (60%) of normal subjects. Similar to the first time assessment group, all subjects of retesting group had inadequate vitamin D levels (< 75 nmol/l). The percentage of 25 (OH) D deficiency among the retesting group subjects is high as well, with the majority of subjects in both the GH sufficient and those with persistent GHD were 25(OH) D deficient (<50 nmol/l).

On the other hand, the majority of our subjects who were 25(OH) D deficient showed PTH levels within the normal range with no significant difference among groups. However, raised PTH> 6.8 pmol/l was noticed in only two subjects: one in the first time assessment (normal) and one in the retesting group (sufficient GH) with a corresponding low 25(OH) D <30nmol/L. In addition, there was a known case of hypoparathyrodism with a low PTH level (0.3 (pmol/l)) in the first time assessment group who had normal GH levels and was excluded from this analysis.

Biochemical markers of bone formation (BAP -OC) were also similar between groups of the first time assessment and retesting, Figures 5-11. However, the medians of OC levels in the first time assessment group were at the lower normal range with (6/15, 40% of naive GHD) and (6/10, 60% of normal) had their OC levels at or below the 10^{th} centiles. Bone resorption marker (CTX) was significantly higher in those with naive GHD compared to normal [2.0 ng/ml (1.4, 3.9) vs. 1.6 ng/ml (0.9, 2.8), respectively, p=0.02], with a median concentration below 10^{th} centiles in the normal group.

Table 5-4 Bone profiles and metabolism markers in the first time assessment and the retesting groups

	First time assessment (n=25)			Retesting (n=9)			Normal ranges
	Naive -GHD (n=15)	Normal (n=10)	P-value	Persistent GHD (n=7)	GH sufficient (n=2)	P-value	
Bone minerals parameters							
Ca (mmol/L)	2.4 (2.2 , 2.9)	2.3 (2.1, 2.9)	0.15	2.4 (2.3, 2.5)	2.2 (2.1, 2.3)	0.13	(2.2, 2.7 mmol/l)
PO4 (mmol/l)	1.4 (1.2, 1.6)	1.3 (0.9, 1.7)	0.33	1.1 (0.9, 1.3)	1.4 (1.4, 1.4)	0.90	(0.9, 1.8 mmol/l)
Mg mmol/L	0.8 (0.7, 1.0)	0.8 (0.7, 1.0)	0.45	0.9 (0.7, 0.9)	0.8 (0.7, 0.8)	0.18	(0.75, 1.00 mmol/L)
PTH (pmol/l)	4.0 (2.3, 5.6)	3.3 (0.3*, 8.7)	0.15 (0.27)*	4.6 (3.4, 6.7)	7.7 (6.2, 9.2)	0.24	(1.0, 6.8 pmol/l)
25 (OH) Vit-D (nmol/l)*	55.7 (23.0, 102.0)	35.5 (20.0, 69.0)	0.16	33.5 (32.0, 55.0)	31.0 (23.0, 39.0)	0.86	(>75 nmol/L)
25(OH) D <50 (n, %)	5, 34%	6,60%	0.24	5, 71%	2,100%	-	
25(OH) D 50-75(n, %)	6,40%	4, 40%	1.00	2,29%	-	-	
25(OH) D >75(n, %)	2,13%	-	-	-	-	-	
No data	2,13%	-	-	-	-	-	
Bone biomarkers+							
BAP (µg/l)	89.5 (17.3, 136.8)	69.9 (0.5, 146.9)	0.25	28.7 (11.8, 81.1)	22.1(15.7, 28.6)	0.61	(48, 121 µg/l)
OC(ng/ml)	50.2 (31.8, 83.6)	46.0 (31.8, 76.5)	0.16	33.6 (25.4, 35.1)	21.7(15.7, 18.4)	0.27	(47, 191 ng/ml)
CTX (ng/ml)	2.0 (1.4, 3.9)	1.6 (0.9, 2.8)	**0.02**	1.1(0.5, 1.6)	1.2 (0.5, 2.0)	0.89	(2, 3.8 ng/ml)

Ca:calcium; PO4: phosphate; Mg:magnisum; PTH: parathyroid hormone; 25 (OH) Vit-D: 25 hydroxy vitamin D; BAP: bone-specific alkaline phosphatase; OC: osteocalcine; CTX: cross linked C-telopeptide of type I collagen

* Known case of hypoparathyrodism was excluded from this analysis (p-value after exclusion).

+ The reference ranges (10th, 90thcentiles) for BAP, OC and CTX were according to local data for children aged from 5years to 16years

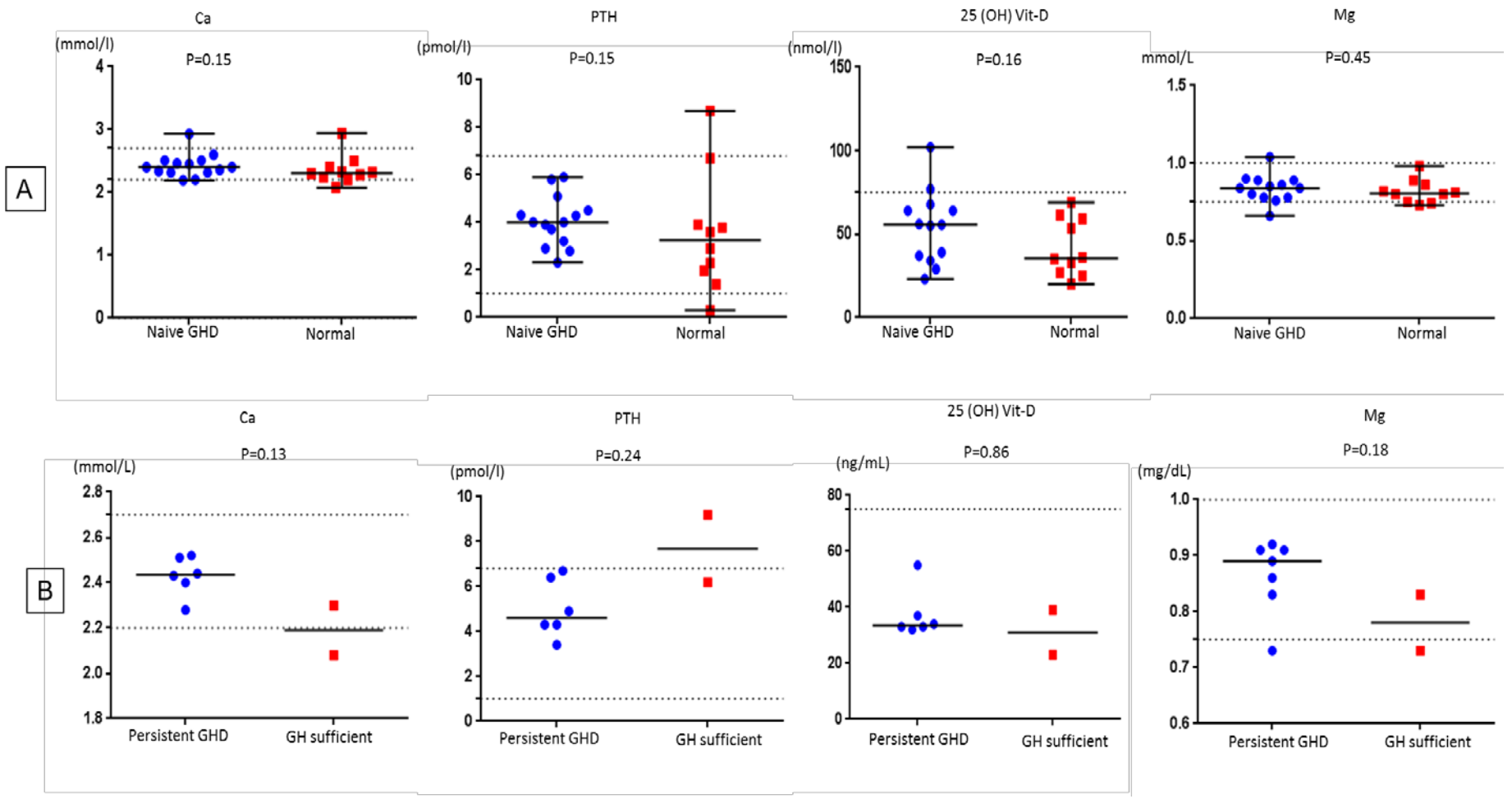

Figure 5-10 Bone profiles and metabolism markers in the first time assessment (A) and the retesting (B) groups.
Individual values with median (range) (blue circle, CO-GHD; Red Square, normal).

Ca: calcium; mg:magnisum; PTH: parathyroid hormone; 25 (OH) Vit-D: 25 hydroxy vitamin D

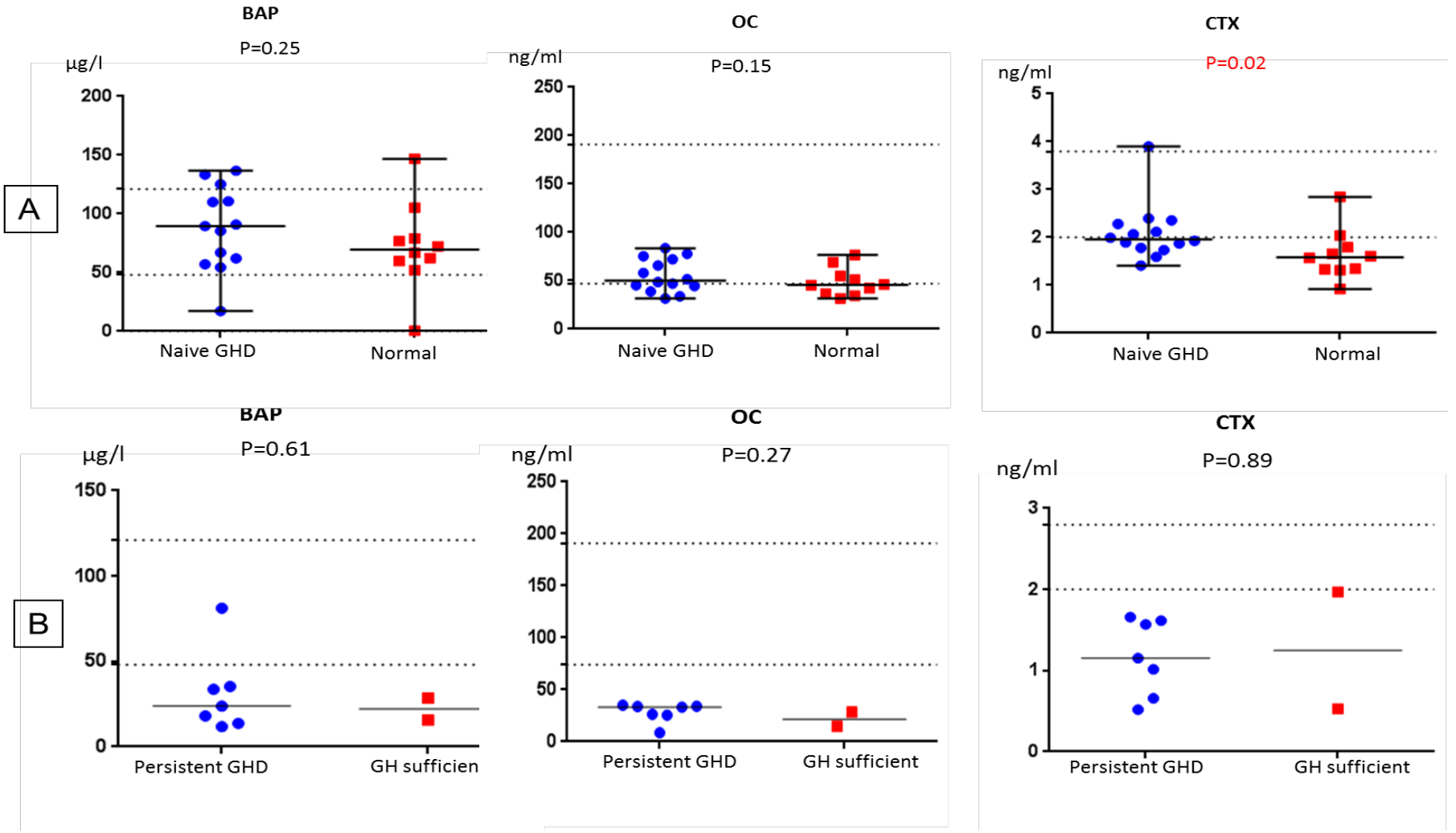

Figure 5-11 Bone biomarkers of the first time assessment (A) and the retesting (B) groups.
Individual values with median (range) (blue circle, CO-GHD; Red Square, normal).

BAP: Bone-specific alkaline phosphatase; OC: osteocalcine; CTX: cross linked C-telopeptide of type I collagen

5.5.8 Muscle strength

The Leonardo mechanograph was used to measure lower limb muscle force, power, velocity, jumping height and efficiency of the movement. For technical reason related to a fault in the cable connects the mechanography platform to the laptop software, therefore mechanography was only available for a few subjects (10/36). Of those, n=8/10 patients were first time assessment; five patient were naive GHD and three normal. Of the remaining 2/10 they were in the retesting group; one of them was reconfirmed persistent GHD and the other was GH sufficient. Individual patient measurements are shown in Table 5-5.

The median of maximum - force (F-max (kN) in naive GHD patients was significantly lower than those who had normal GH levels [0.5 (0.3, 2.8) vs. 2.7 (2.2, 3.3) respectively, p= 0.03] which was proportional to tibia muscle CSA, but not to LM. Scatterplot of maximum – force, GH peak, tibia muscle CSA and LM in naive GHD and normal of first time assessment group are illustrated in Figures 5-12, 5-13, 5- 14. There were no significant differences in the other mechanography parameter measurements although the naive GHD group showed lower medians in all of the measured parameters. To assess the muscle–bone relationship, we correlated mechanogrphic data with tibia pQCT data. Tibia muscle CSA was correlated positively with maximal power (r= 0.85, p=0.01), Figure 5-15.

With regards to the two adolescents' patients who were CO-GHD, from Table 5-5, the individual with persistent GHD, mechanogroph measurements were lower than the patient who was GH sufficient.

Table 5-5 Individual data of Mechanography measurements and the related clinical and body composition data.

Subject	Age (Yrs)	Sex	GH-peak (mg/l)	Jump height (m)	V-max(m/s)	EFI%	Fmax(tot)kN	P-max(tot)kW	Efficiency %	TB-LM (Kg)	Tibia-Muscle CSA (mm^2)
Pt-1[1]	11.0	M	2.3	0.48	2.51	117	0.95	1.9	120	32.5	4889.2
Pt-2[1]	15.0	M	0.9	0.31	2.01	83	1.92	2.83	73	36.9	5516.0
Pt-3[1]	5.6	M	2.4	0.12	1.15	72	0.4	0.3	63	11.4	1989.7
Pt-4[1]	10.9	M	4.2	0.48	1.61	92	0.48	0.53	77	22.9	4070.0
Pt-5[1]	8.2	M	3.1	0.2	1.67	94	0.5	0.54	77	11.9	2170.5
Pt-6[2]	5.8	F	6.7	0.16	1.55	97	2.71	0.50	86	13.3	368.5
Pt-7[2]	9.0	M	6.7	0.26	2.02	115	3.33	0.91	83	17.7	-
Pt-8[2]	16.5	M	8	0.37	2.30	82	2.29	1.53	86	29.6	3524.7
Pt-9[3]	18.6	F	3.3	0.19	1.74	65	1.11	1.42	66	28.0	4003.7
Pt-10[4]	16.3	F	10.2	0.28	2.03	79	2.4	2.45	79	38.9	5247.2
Median (range) (naive GHD) n=5	10.9 (5.6-12.0)	-	-	0.31 (0.12, 0.48)	1.6 (1.1, 2.5)	92.5 (72.0, 117.0)	0.5 (0.3, 2.8)	0.5 (0.3, 2.8)	77.0 (63.0, 120)	22.9 (11.4, 36.9)	4070.0 (1989.8, 5516.0)
Median(range) (Normal) n=3	9.0 (5.8-16.5)	-	-	0.26 (0.16, 0.37)	2.0 (1.5, 2.3)	97.0 (82.0, 115.0)	2.7 (2.29, 3.33)	0.9 (0.5, 1.5)	86.0 (83.0, 86.0)	17.7 (13.3, 29.9)	1946.6 (368.5, 3524.8)
P-value	0.91	-	-	0.76	0.76	0.74	**0.03**	1.00	0.32	0.99	0.25

1: naive GHD; 2: normal; 3: persistent GHD; 4: GH sufficient

V-max (m/s: Maximum-velocity; EFI %: Esslinger fitness index; F-max (tot) (kN): maximum –force; P-max (tot) kW: maximum-power; TB-LM: total body lean mass; tibia muscle CSA: tibia muscles cross sectional area

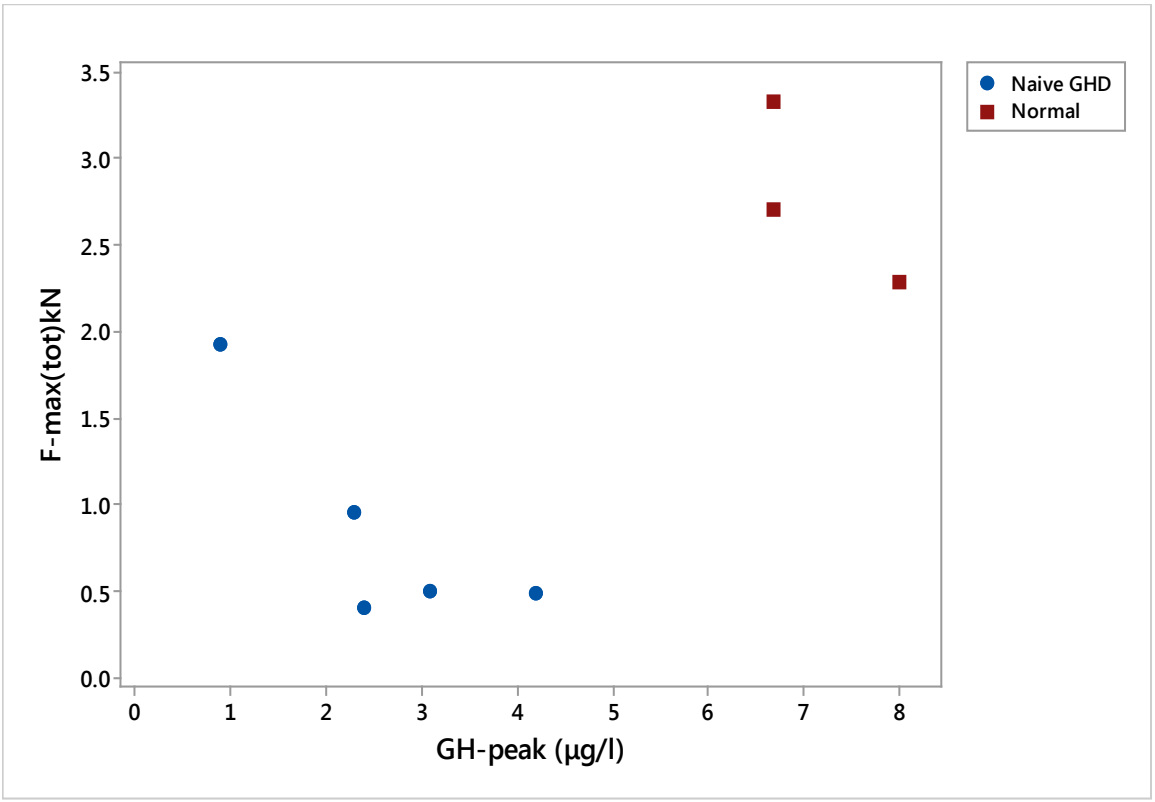

Figure 5-12 Scatterplot of maximum - force and GH peak in naive GHD and normal of the first time assessment group

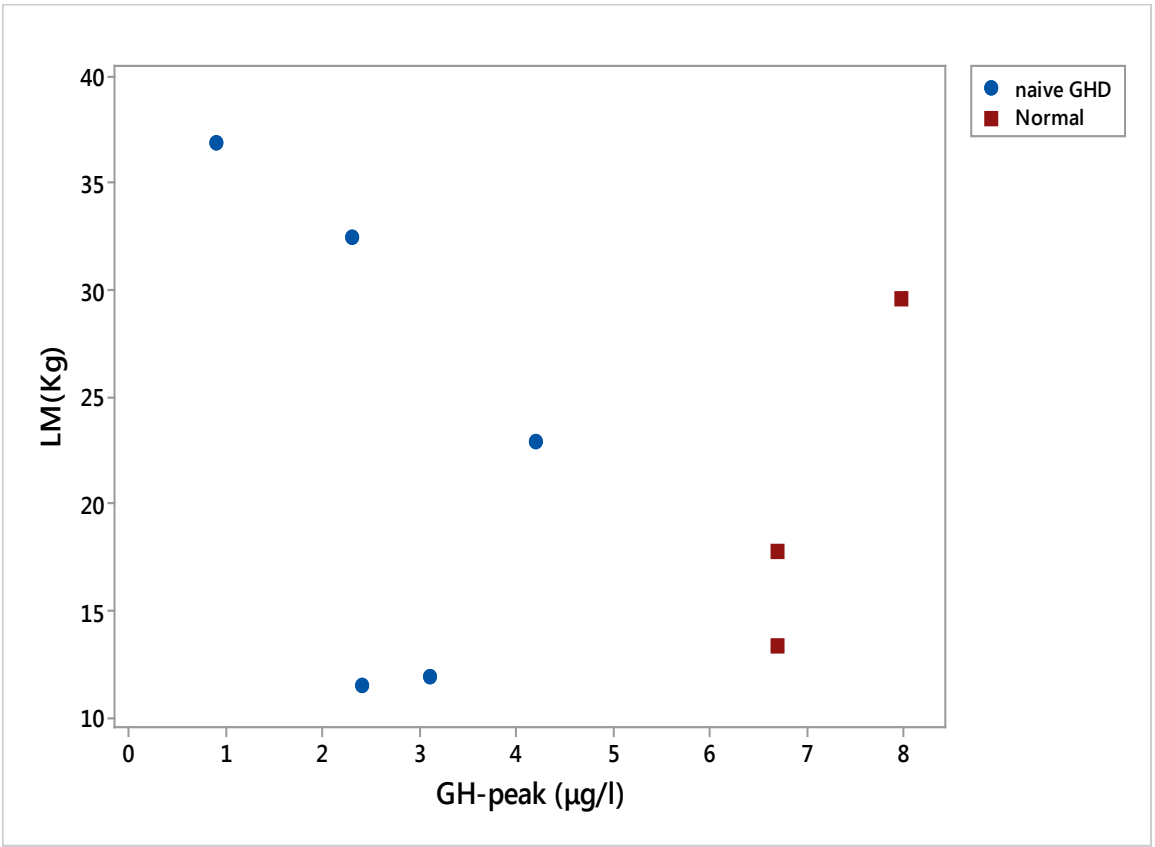

Figure 5-13 Scatterplot of LM and GH-peak in naive GHD and normal of the first time assessment group

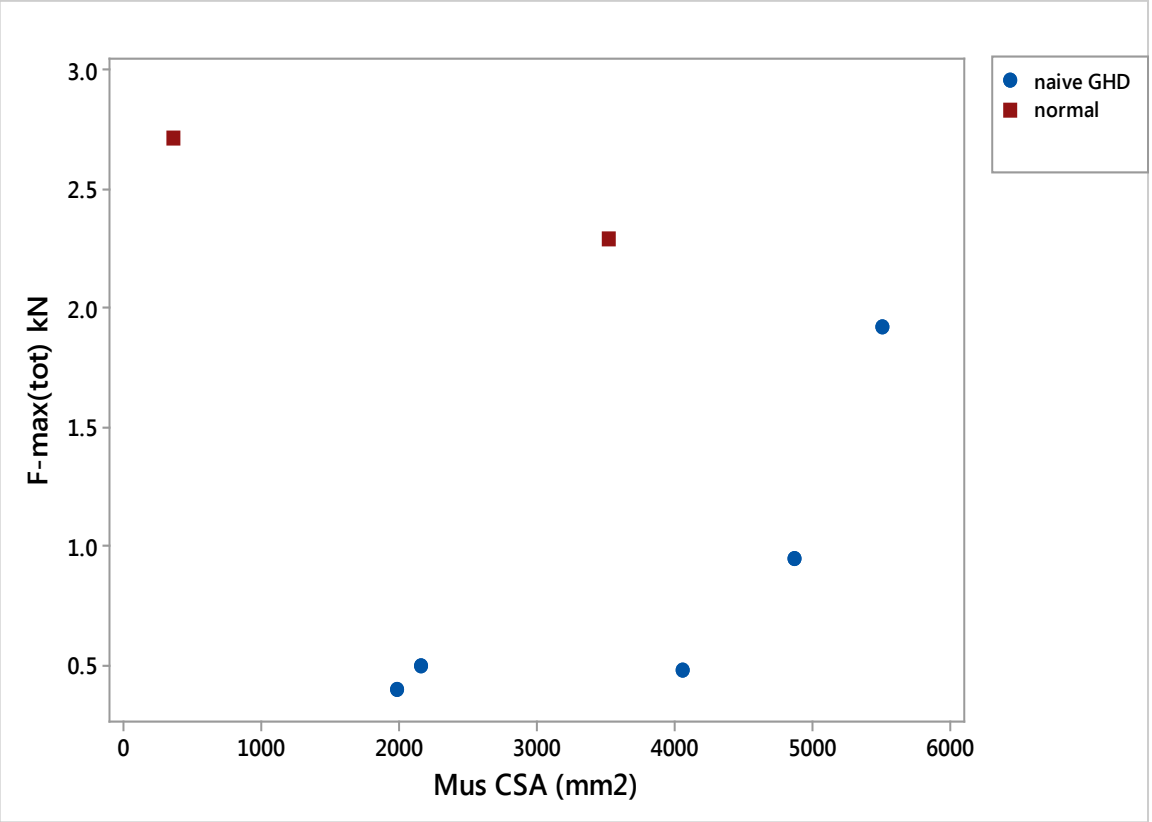

Figure 5-14 Scatterplot of maximum - force and tibia muscle cross sectional area in naive GHD and normal of the first time assessment group

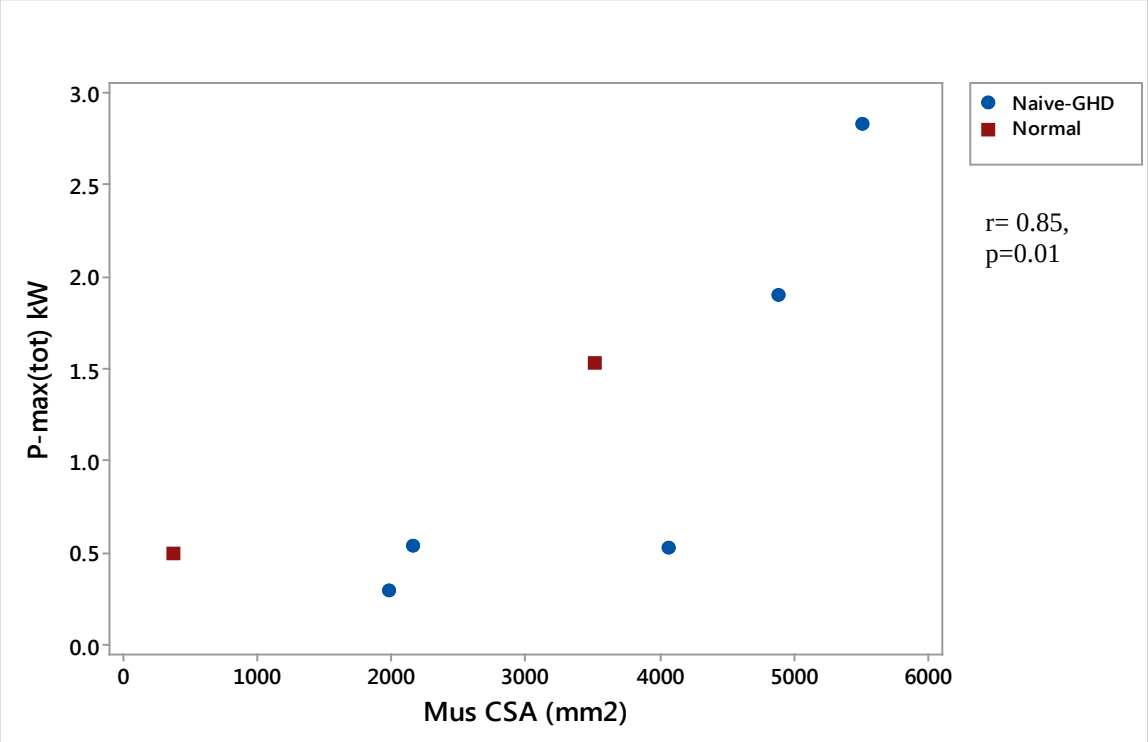

Figure 5-15 Scatterplot of the correlation between maximum-power (kN) and tibia muscle CSA

5.5.9 Correlation between DXA and pQCT tibia parameters with clinical and anthropometric data

Age and anthropometric parameters (height-weight-BMI) were significantly related to all of the markers of bone density and geometry in the first time assessment group, but the relations became attenuated in the retesting group.

Both FM and LM were positively correlated with TB/LS BMD, BMC and BA of the first time assessment group, when only LM was correlated positively with TB/LS BMC and BA of the retesting group. Our data showed strong positive correlations (r ranges (0.56, 0.98), all $p<0.05$) between DXA derivative parameters (TB/LS-BMD-BMC-BA-LM-FM) and tibia bone geometry (cortical thickness, and periosteal circumferences, cortical bone area and SSI) in the first time assessment group, but these significant relationships were weak (not significant) in the retesting group (all $p>0.05$). None of these DXA parameters showed any relation with tibia-pQCT density parameters (total density -trabecular density- cortical density) (all $P>0.05$) in both groups.

GH peak levels did not correlated to bone density and body composition parameters as measured by DXA and pQCT in both first time assessment and retesting groups, but IGF-1 levels in the first time assessment were correlated significantly positively with TB/LS BMD, BMC and LM of DXA measurement and only with cortical thickness, periosteal circumference, cortical density, cortical CSA and muscle CSA of pQCT (all $p<0.01$).

In the first time assessment, GH peak levels was correlated negatively with CTX levels ($r=-0.46$, $p=0.02$) Figure 5-16, when among the retesting group, CTX levels was correlated positively with duration from discontinuation rhGH to retesting ($r= 0.731$, $p=0.04$), Figure 5-17.

No significant correlations were observed between 25 (OH) Vit-D with Ca, PTH and bone biomarkers in both groups. However, PTH levels showed significant positive correlations with CTX in both first time assessment ($r=0.46$, $p=0.02$) and retesting groups ($r=0.77$, $p=0.02$), Figures 5-18, 5-19**.**

No significant correlations were found between levels of bone profile parameters and metabolism biomarkers with either scanning data of bone or body composition.

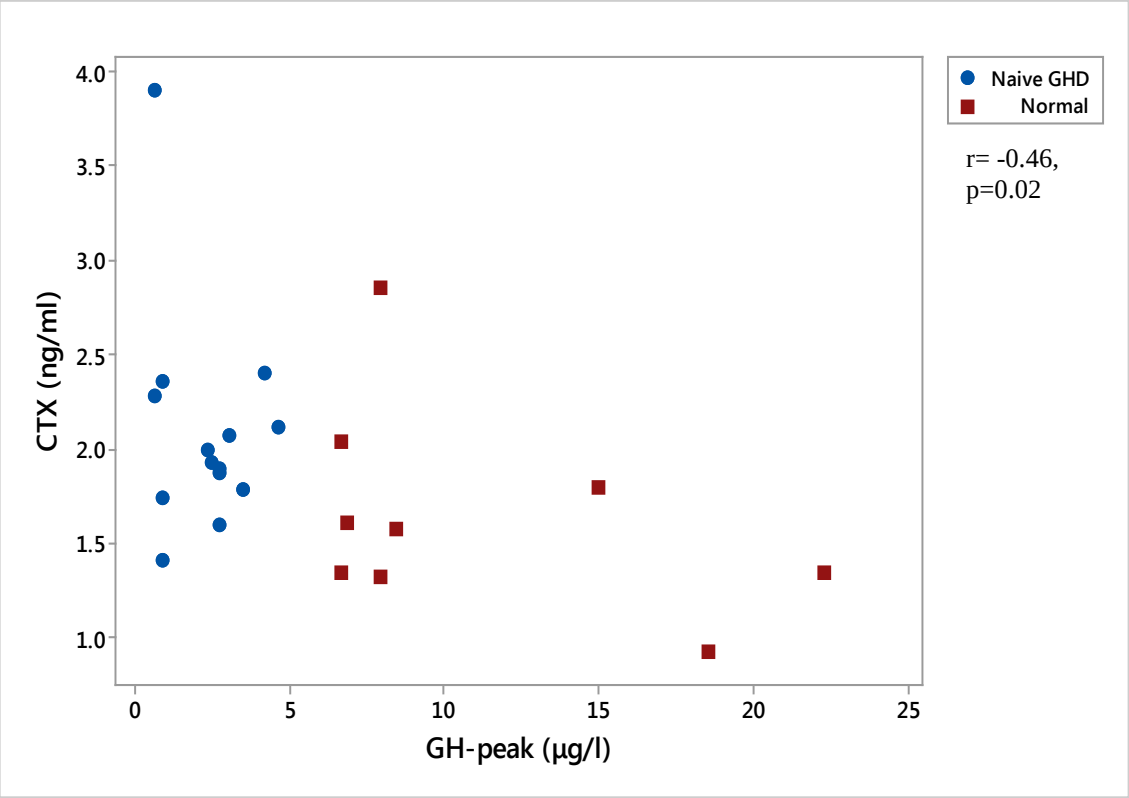

Figure 5-16 Scatterplots showing correlation between GH peak levels and CTX levels in in the first time assessment group

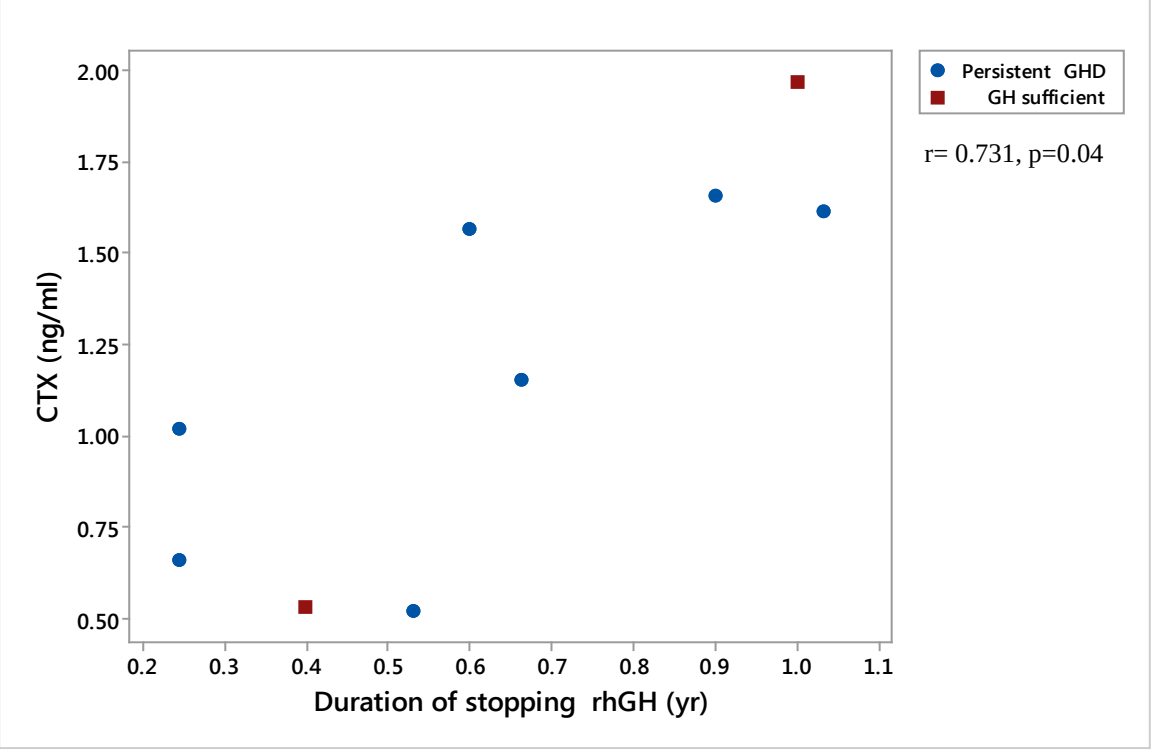

Figure 5-17 Scatterplots showing correlation between duration of stopping rhGH and CTX levels at retesting

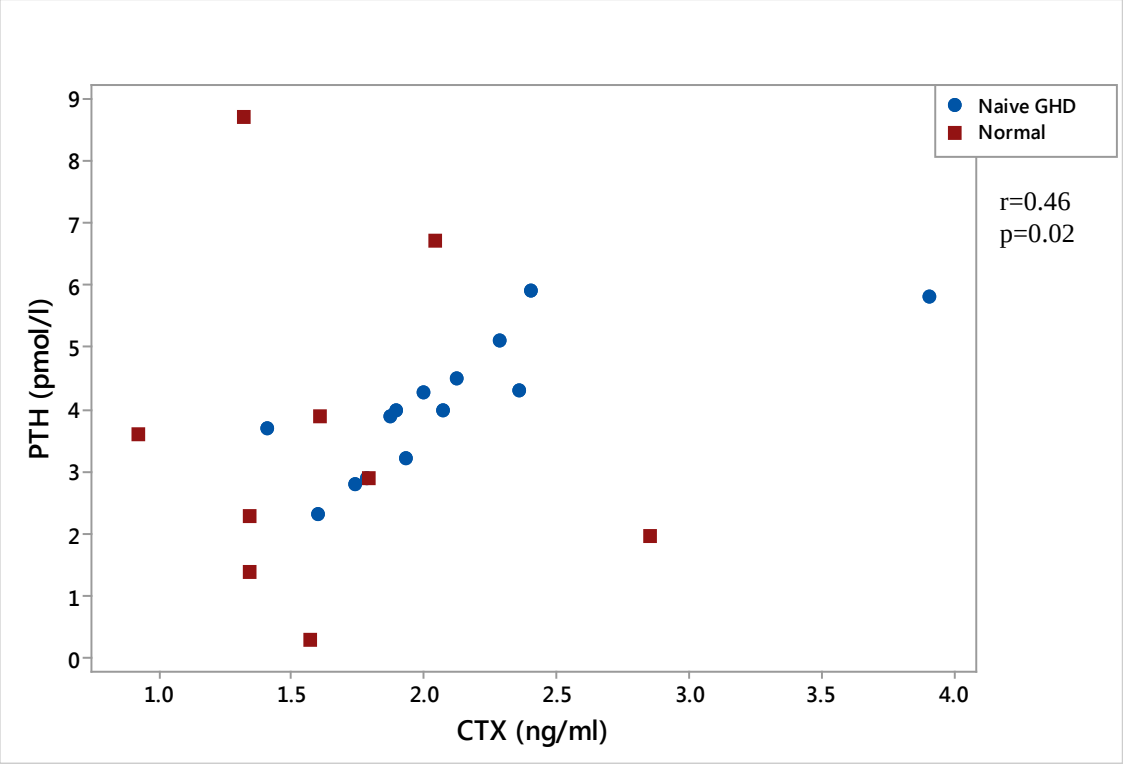

Figure 5-18 Scatterplots showing correlation between PTH levels and CTX levels in the first time assessment group

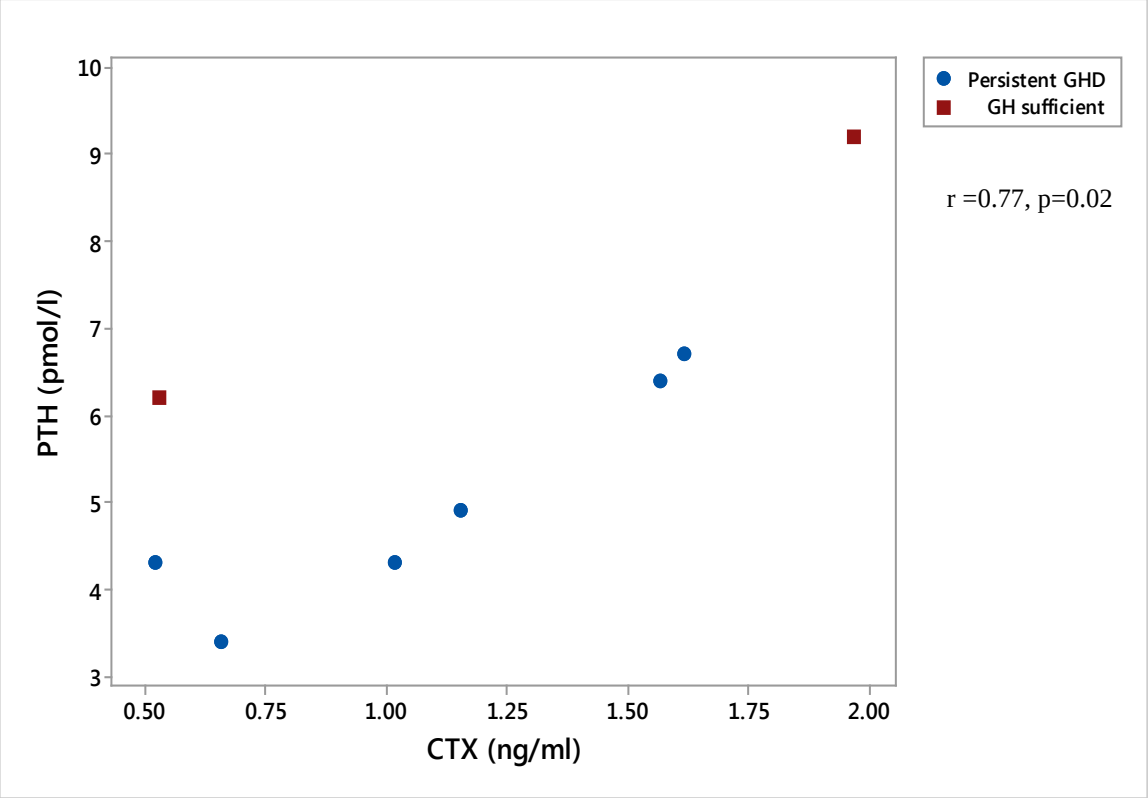

Figure 5-19 Scatterplots showing correlations between PTH levels and CTX levels in the retesting group

5.6 Discussion

In this cross-sectional study, we showed that bone mass and body composition characteristics using DXA and pQCT images in subjects with CO-GHD at the time of first evaluation and retesting after final height are strikingly similar to those who have normal GH secretion at either time point. Considering various correction methods, no major differences in areal and volumetric total body and lumber spinal bone density have been found between children with short stature as a result of GHD at baseline and prior to receiving rhGH or at retesting after withdrawal of rhGH at final height when compared to normal GH levels matched groups, Our results are consistent with previously published data (89,223,232,413). On the other hand, pQCT based studies found children and adults with CO-GHD to have low cortical thickness but normal volumetric density (235,238). Our results, however, showed similar tibia bone geometry and density between naive GHD and normal in the first time assessment group, but persistent GHD in the retesting group had larger periosteal circumferences and lower cortical density without significant differences in cortical thickness compared to GH sufficient, which were not evident when adjusted for height and age Z scores. However, we postulated this mild differences may arise from gender dimorphism during puberty maturation between compared groups of retesting n=6 (3males-3 females) persistent GHD vs. n=3 (3 females) GH sufficient (414).

In fact, measurement of bone density in children and adolescents is less standardized than in adults. DXA as a projectional technique has several limitations for the assessment of bone status in children. The major limitation of DXA is size dependence of the density measuring areal BMD in the two-dimensional area of a three-dimensional bone structure, disregarding bone depth (54). Other DXA limitations are: DXA cannot differentiate between cortical and trabecular bone, and imaging artifacts can also cause inaccuracies of DXA measurements (415). On the other hand, pQCT has mostly been carried out as a research tool allowing for the measurement of true volumetric BMD at peripheral sites (distal raids and distal tibia). In contrast to DXA, pQCT can distinguish between cortical bone from trabecular bone (405). Technically, pQCT is similar to or worse than DXA, as patient movement during scanning in particular in very young age can cause errors in locating the measurement site affecting reproducibility (405,415). In addition, the most challenging limitation of pQCT is underestimation of cortical vBMD when the cortical thickness is below 2mm which is known as the partial volume effect and happens when a voxel in the image represents more than one tissue type (overlapping bone and soft tissue) (405). Therefore, pQCT tibia measurement is suggested to be more preferred in children than the radius to overcome parietal volume effects and is less susceptible to movement artifacts (416). pQCT has also limitations in locating scanning sites, in particular for longitudinal studies. In growing children, it can be difficult to obtain repeated pQCT measurement of the same location in paediatric longitudinal studies due to changing size of the metaphysis with growth and inconsistence landmarks for reference line placement (406,417). To date, there is no established reference data for tibia pQCT measurement in children due to a lack of a sufficiently large representative sample

of healthy children, with the added difficulty in using various scanner device models and software versions (406,416).

Hence, the impact of CO-GHD on bone of children and adolescents has been a challenge to discern. Considering limitations of both DXA and pQCT with incomplete fracture data in CO-GHD, new insights into the relationships between cortical and trabecular bone macrostructure, microarchitecture in CO-GHD are emerging with advances in imaging techniques. Evolving data from animal and human studies demonstrated that GHD may impair bone microarchitecture based on histomorphometric findings supported by microCT images (235,238,418). Mice studies revealed that GHD results in a deterioration of bone size, microarchitecture but not mechanical properties (418). A recent study using advanced micro-MRI images to describe detailed bone microarchitecture of CO-GHD revealed a significantly lower ratio of apparent bone volume to total volume (appBV/TV) and apparent trabecular number (appTbN) in GHD compared to age-matched control group (239). Further studies using advance imaging techniques are needed to explore bone microstructure in CO-GHD.

It has been long recognized that adaptation of bone morphology is dominated by mechanical loads placed on the skeleton (408). Consequently, it was assumed that changes in muscle mass and function as a result of GHD may play a crucial role in determining bone density of subjects with GHD (419). Accordingly, it has been suggested that the deficits in somatic development with lower LM in children with GHD is a potential confounding factor in the determination of bone density and mass (381,420). Indeed, the expected body composition in patients with GHD is an increased proportion of FM to LM as it was frequently reported in adult onset GHD (421), adults with childhood-onset GHD (233,245), but less frequently in childhood studies (88,422). Although we did not show any differences in either LM or FM between studied groups, theses compartments were positively associated with TB/LS BMC, BMD and BA and with only with tibia geometry but not tibia density.

In terms of muscle strength , in previous studies, a lower maximum isotonic strength as measured by hand grip force has been documented in young adults with CO-GHD and adult- onset GHD (225,423,424), but still remains to be completely elucidated in children with CO-GHD. In fact, assessment of muscle function and strength by hand grip force has limited relevance for the examination of muscle force because it only assesses isometric force at the upper extremity (non-weight-bearing part of the body). Leonardo jumping mechanography is a new reliable technique of evaluation muscle function and strength in children, adolescents and adults (75-78). Unlike isometric force measurements, mechanography allows to measure maximum muscle force and peak power using various tests (76). Evidence suggests that there is a positive correlation between maximum force and tibia bone parameters as measured by pQCT, and shows that the maximum force predicted 84% of tibia BMC of children and adults (71). Currently, there is no consistent paediatric reference data, but there are several published reference data intended to assist clinicians in the assessment of muscle function by jumping mechanography in Caucasian children and

adolescents (425,426). Although small data, a significant lower maximum-force as measured by mechanography was observed in naive GHD compared to normal in the first time assessment group, when the only assessed two subjects among retesting group, the persistent GHD showed lower muscle force and power compared to the subject with GH sufficient. Up to our knowledge, few studies have analysed the connection between bone and muscle strength in adults with GHD (427), with no existing data targeting children and adolescents with CO-GHD. Although were not able to provide complete data on the muscle–bone interaction in our cohort, it can nevertheless be interpreted in the framework of the mechanostat theory.

Our data also showed a high percentage of the first time and the retesting groups had abnormal levels of 25(OH) D according to Endocrine Society recommendations (428). However, to date, there is limited data on which levels of 25(OH) D are associated with subtle abnormalities of bone density, metabolism and neuromuscular function (60). Although the relation between 25(OH) D, PTH, and GH/IGF-1 axis is well documented (429-431), this relationship is not clearly established in subjects with GHD. It has been suggested that GH/IGF-1 increases 1α-hydroxylase activity in the kidney, thereby increasing 1, 25-dihydroxyvitmin D levels (155,432). Recent studies demonstrated a high prevalence of hypovitaminosis D (<30 ng/mL) in children (433) and adults with GHD(434). In the present study, the majority of subjects had inadequate vitamin D levels whether they had GHD or normal GH levels, with no significant correlations with either GH peak or IGF-1 levels.

On the other hand, there is evidence suggesting an interactive relationship between PTH and GH on bone: PTH plays an important catabolic role on bone metabolism through stimulating osteoclast differentiation and increasing bone resorption and remodelling (62,63) and GH may have a regulatory role in modulating PTH secretion and circadian rhythm (44,165). In the context of GHD, studies of adults with GHD assumed PTH may underlie bone deficits in GHD through reducing bone turnover and increasing bone resorptive activity (91,435). In addition, it was suggested that subjects with GHD showed a decrease in organ sensitivity to PTH leading to a state of PTH resistance without a significant change in concentrations (436). In support of this concept, our analysis showed a significant positive correlation between PTH and CTX levels and that was more pronounced in those with GHD among both groups. In addition CTX levels showed positive correlation with duration of discontinuation rhGH after final height in retesting group which may relate to increases PTH actions after withdrawal rhGH.

There is also strong evidence from in vivo and vitro studies reporting that GH increases bone turnover by acting directly and indirectly on target bone cells (163).So far, data is not all conclusive whether CO-GHD results in imbalanced bone turnover which ultimately could cause low bone mass (92). It is well documented that adults with GHD have low bone turnover predisposing them to osteoporosis (437). Intervention studies showed that rhGH replacement increases biochemical

markers of bone turnover in the normal population (438) children with GHD during childhood and at final height when restarting rhGH (223,410). Although the medians of formation markers were not different between groups of the first time assessment and retesting, it appears that in terms of the percentage of higher bone resorptive and lower formation activities was more pronounced in GHD patients. As no significant correlations between both GH/IGF-1 and bone turnover biomarkers, except CTX levels in the first time assessment group, were found in our data, it could indicate that factors other than GH statue may play a role.

Although we must take into account the limitations of this study that we cannot gain any insight on bone pathophysiology in GHD from the small size cross sectional data, our data may suggest the possibilities that may be needed to further investigated in larger studies.

5.7 Conclusion

The present study provides additional evidence that bone density and body composition were not affected in children and adolescents with CO-GHD either at time of first assessment or on retesting at final height as measured by DXA and pQCT using various corrections methods. Muscle strength decline may be an issue connected with the bone health in subjects with CO-GHD. Our results also suggest that serum PTH may be an important determinant of bone metabolism in subjects with CO-GHD. These findings may explain a possible underlying mechanism for the impact of CO-GHD on bone health. However, a large-scale study is required to verify our findings to derive a more accurate and trustworthy conclusion.

CHAPTER 6

Metabolic Parameters and Glucose Homeostasis in Children and Adolescents with Childhood-Onset Growth Hormone Deficiency at Time of Initial Evaluation and Retesting at Final Height

6.1 Abstract

Background: It is well known that growth hormone (GH) has several functions and effects, involving bone, body composition, lipids and glucose homeostasis. However, the complex interplay between these parameters is rather poorly studied in children with childhood-onset-GH deficiency (CO-GHD).

Aim: To investigate lipids, adipokines (leptin- adiponectin- resistin) and glucose homeostasis and their relationship with bone and body composition in children and adolescents with CO-GHD at time of initial evaluation and retesting at final height.

Study population and methods: A cross-sectional study of children undergoing GH stimulation tests for investigation of short stature (total –25, GH deficiency identified –15, median age (range) 10.9years (5.6-16.0)) and adolescents with CO-GHD undergoing biochemical revaluation of the GH axis at final height after withdrawal of GH therapy (total- 11, persistent GHD-7, age 16.7years (14.9-18.6)).

Results: At the time of initial evaluation and retesting, lipid profiles, adipokines and glucose homeostasis were not different between those with GH deficiency and those who had normal GH levels across groups. Leptin levels in both groups correlated positively with fat mass ($r=0.9$, $p<0.01$), and with osteocalcin positively at initial evaluation ($r=0.51$, $p<0.01$) but inversely at retesting ($r=-0.91$, $p<0.01$). In the retesting group, those who were older at the time of diagnosis of CO-GHD with a shorter duration of GH therapy were more likely to have a higher cholesterol ($r=0.9$, $p<0.001$) and leptin ($r=0.8$, $p<0.001$), with a lower osteoclacin ($r=-0.7$, $p=0.01$) at final height.

Conclusion: Metabolic profiles and glucose homeostasis were not significantly different between those with GH deficiency and those with normal GH levels at time of initial evaluation and retesting at final height. Timing and duration of childhood treatment may influence adiposity parameters and bone formation biomarkers in adolescents with CO-GHD. Differences in relationship between leptin and osteoclacin at time of initial diagnosis and at the time of retesting may be related to active growth. Further studies are still required to clarify the relationship between adipokines, metabolic profiles and bone in subjects with CO-GHD.

6.2 Introduction

The main role of growth hormone (GH) in growing children and adolescents is to promote linear growth and maintain bone health and body composition (362,439). Nevertheless, it is well known that GH brings about a large number of metabolic effects, involving lipid profiles, adipokines and glucose homeostasis (133). These issues have become the focus of research in recent years. Studies have demonstrated a slight increase in unfavourable lipid profiles in children with childhood onset GH-deficiency (CO-GHD), both at the time of diagnosis (440,441) and in GH-deficient adolescents after discontinuation of recombinant human GH (rhGH) treatment (268,442). These parameters have been shown to improve after rhGH replacement therapy (440-442).

While it is well documented that adult subjects with GHD are more likely to be insulin resistant (443), children with CO-GHD are known to be more insulin sensitive at time of first diagnosis (444) and after withdrawal of rhGH at final height (244). However, it is not clear whether GHD itself, body composition and adiposity or both are responsible for glucose homeostasis changes in these subjects. Adipokines (particular leptin, adiponectin and resistin) have been suggested to be involved in insulin sensitivity status and in the regulation of glucose homeostasis and energy metabolism (144). Interestingly, recent studies provide evidence that adipokines might also participate in bone metabolism through different mechanisms (445). Several reports indicate leptin, which correlates positively with BMI and fat mass and negatively with insulin sensitivity, may also play a role in bone mass regulation by stimulating osteoblastic activity and controlling osteoclastogenesis (160). Adiponectin has been proposed to play an important role in the regulation of energy homeostasis and insulin sensitivity (446), but its role in bone density and metabolism is unclear (158,447). Resistin, another cytokine, has been implicated in the regulation of inflammatory processes and insulin sensitivity (448), but debate is still ongoing with its exact biological functions in humans and animals studies. Resistin in rodents is clearly directly linked with insulin resistance, when human resistin is shown to have a significant role in inflammation processes which may be indirectly causing insulin resistance (449). So far, there are a few studies evaluating adipokines in children and adolescents with CO-GHD (440,449-452), but no study has been carried out showing the relationship between adipokines and bone metabolism in these patients.

Therefore, we aim to investigate lipid glucose homeostasis, adipokines levels and their relationship with bone metabolism markers and body composition in children and adolescents with CO-GHD at time of initial evaluation or retesting at final height.

6.3 Subjects and Methods

6.3.1 Patients

We studied 25 children undergoing GH stimulation tests for investigation of short stature (first time assessment group), aged median (range) 10.9 years (5.6-16.0), and 11 adolescents with CO-GHD who had biochemical retesting at final height after withdrawal of childhood rhGH therapy (retesting group), aged 16.7 years (14.9-18.6). The inclusion and exclusion criteria of this study were described in chapter 5. Of the 25 first time assessments, 15 were confirmed GH deficient (naive-GHD) (GH peak on stimulation tests <6.6 μg/l), and seven of the 11-retesting group had persistent GHD (GH peak <5 μg/l and or low IGF1 < -2 SD for age references). Obesity was defined as BMI SDS > +2 SDS above the mean using UK 1990 growth references, and underweight was defined as BMI SDS < -2 SDS (384,453).
Only subjects who had fasting blood samples taken and tested were involved in this analysis.

6.3.2 Hormone and biochemical assays

Blood samples for metabolic parameters were taken after overnight fasting, on the day of the stimulation test before starting GH therapy. Lipid profiles (total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides (TG)) levels were measured immediately after blood sampling in a biochemistry laboratory in RHC with standard methods and reference ranges from CALIPER (411).
Leptin levels were measured by ELISA according to the manufacturer's instructions (Linco Research, Inc., St Charles, MO, USA) with intra-assay CV% values of 2.0–2.5%. Adiponectin levels were measured by ELISA according to the manufacturer's instructions (Linco Research, Inc.) with inter-assay CV values of 20.3 to 0.9%. Resistin concentration was assayed with an enzyme-linked immunosorbent assay kit (EZHR-95K, Linco Research, St Charles, MO). The sensitivity of the assay was 0.16 ng/mL, with intra-assay CV of 3.1% to 15.2%. As serum adipokine levels are dependent on the amount of adipose tissue, adipokine levels were adjusted for fat mass (FM) by dividing the measured concentration by FM as measured by DXA (chapter-5).
The serum free fatty acid (FFA) concentration was determined using the ACS.ACOD method (Wako Pure Chemical Industries, Osaka, Japan) with intra-assay coefficients and a variation of less than 1.5 %. Serum insulin was measured by ELISA enzymeimmunoassay (DRG Human Insulin EIA-2935, Germany) with intra essay CV of 1.4-1.7%. All ELISAs were performed by Mr Martin McMillan in the Department of Child Health at RHC, Glasgow. Fasting insulin normal reference values by age and weight were according to (453) and (454). The insulin resistance was estimated using the homeostasis model assessment insulin resistance index (HOMA-IR), and using the following formula: fasting serum insulin (μU/ml) × fasting plasma glucose (mmol/l)/22.5 (455). HOMA-IR estimates steady state beta pancreatic cell function and reflects target-tissue insulin

sensitivity to insulin as percentage from a normal reference population. This measure is simple, minimally invasive and well correspond to other surrogate markers of insulin resistance such as QUICKI, hyperinsulinaemic clamp and the oral glucose tolerance test (456) with some cautions for its implications for clinical practice (457,458). There is currently no consensus as to the optimal cut-offs for HOMA-IR amongst children and adolescents. In this study HOMA-IR values of > 2.2 (age 5-8), >3.3 (age 9-11) and > 4.5 (age 12-19) were chosen as an indicator of reduced insulin sensitivity (453,454).

6.3.3 Bone and body composition

Bone turnover markers and body composition data were described in chapter-5.

6.4 Statistical Analysis

Data were reported as the median and range (minimum-maximum). Differences between medians were analysed by the Mann- Whitney U-test (nonparametric test). Correlations among continuous variables without normal distribution were determined by using the Spearman's test. Linear regression analysis was used to test the relationships of adipokines with other variables (focused significance cutoff: <0.05 for univariate analysis) after adjusting for FM. P< 0.05 was considered statistically significant. All graphs were performed using GraphPad Prism 6 software (GraphPad Software, San Diego, CA, USA).

6.5 Results

6.5.1 General characteristics

Auxological and clinical parameters of both groups are reported in Table 6-1. Both groups were similar in age, height and BMI (p values were not significant). However, two subjects with naive GHD were obese (BMI SDS: 2.9, 3.0), and one subject with normal GH levels was under weight (BMI SDS: -2.4). The majority of first time assessment were prepubertal (tanner stage I) 10/15(67%) of naive GHD; 5/10(50%) of normal subjects.
No differences were recorded in the age of diagnosis, duration of treatment and time off treatment in the retesting group between those who had persistent GHD and those who were now GH sufficient.

Table 6-1 Auxological and clinical characteristics of the first time assessment and the retesting groups.

	First time assessment (n=25)		P-value	Retesting (n=9)		P-value
	Naive-GHD (n=15)	Normal (n=10)		Persistent GHD (n=7)	GH-sufficient (n=2)	
M/F	13/2	7/3	0.30	3/4	0/2	0.50
Age(yrs)	10.9 (5.6, 15.2)	12.1 (5.8, 16.5)	0.90	16.6 (14.9, 18.6)	16.8(16.3-20.4)	0.73
Anthropometry						
Height (cm)	129.0 (97,152.2)	130.1(96.1, 153.3)	0.76	158.7(152.7, 179)	153.5(145.3, 166.4)	0.35
Height -SDS	-2.5 (-3.4, 1.3)	-2.2 (-4.6, -0.1)	0.51	-1.2 (-1.9, 1.2)	-1.6(-3.0, 0.5)	0.50
Weight (kg)	26.5 (15, 71.4)	29.3 (13.3, 56.7)	0.94	60.6(45.6, 71.2)	56.0(37.6, 66.7)	0.63
Weight-SDS	-1.8 (-3.6, 1.9)	-1.3 (-4.7, 0.7)	0.93	0.6 (-1.8, 1.4)	0.0(-3.2, 1.1)	0.63
BMI	16.5 (14.2, 32.3)	17.2(14.4, 24.7)	0.97	22.4(18.8, 28.0)	23.8(17.8, 24.1)	0.65
BMI-SDS	0.0(-1.8, 3.0)	0.0 (-2.4, 1.6)	0.57	0.9 (-1.1, 2.0)	1.0(-1.4, 1.1)	0.59
Biochemical data						
GH-peak(µg/l)	2.6 (0.7, 4.7)	8.0 (6.7, 22.3)	<0.01	2.0 (0.1, 3.8)	8.3 (6.4, 10.2)	0.05
IGF1 levels(ng/ml)	65.0 (14.0, 433.0)	85.5(28.0, 295.0)	0.52	141.0 (18.0, 294.0)	241.5(117, 327.0)	0.28
IGF1 levels SDS	-3.2 (<-5.0, 0.3)	-2.0 (-4.5, -0.9)	0.72	-3.2 (<-5.0, -1.3)	-2.0 (-3.5, -0.9)	0.28
Tanner stages n (%)						
I	10 (66)	5 (50)	0.44			
II	3 (20)	2 (20)	0.95			
III	2 (20)	2 (20)	0.94	-	-	
IV	0	1 (10)	0.20			
V	0	0				
Retesting data						
Age of diagnosis (yr)				9.5 (2.6, 10.3)	9.2 (7.0, 11.4)	0.56
Age at starting rhGH(yr)				10.3 (7.1, 13.6)	9.2 (7.0, 11.4)	0.56
Duration of rhGH (yr)				4.7 (2.9, 7.8)	7.3 (4.3, 10.2)	0.68
Age at stopping rhGH(yr)				15.9 (14.4, 17.9)	16.4 (15.7, 17.0)	0.86
Duration of stopping rhGH (yr)				0.6 (0.2, 1.0)	0.7 (0.4, 1.0)	0.86

Data are reported as median and (range)

6.5.2 Lipid profiles

From Table 6-2, at time of initial evaluation and retesting, no statistical significant differences were found in lipid profiles: total cholesterol, LHD, LDL, TG, CHOL/HDL ratio and FFA between those with GH deficiency and those who had normal GH levels in both studied groups.
Individual data of lipid profiles in the first time assessment and the retesting groups are illustrated in Figures 6-1, 6-2**.**

Table 6-2 Lipid profiles, adipokines and glucose homeostasis parameters in the first time assessment and the retesting groups.
Data are reported as median and (range)

	First time assessment		P-value	Retesting		P-value	Normal ranges
	Naive-GHD (n=15)	Normal (n=10)		Persistent GHD (n=7)	GH-sufficient (n=2)		
Lipid profiles							
T-CHOL (mmol/L)	3.8(2.8, 5.2)	4.4(2.9, 5.2)	0.89	4.0(3.3, 4.8)	3.7(3.2, 4.1)	0.50	(2.97–4.72)
HDL (mmol/L)	1.5(1.1, 2.1)	1.3(0.9, 2.2)	0.18	1.1(0.9, 2.1)	1.1(0.9, 1.3)	0.99	(0.73–1.87)
LDL (mmol/L)	1.9(1.4, 3.2)	2.3(1.7, 3.4)	0.12	2.1(1.6, 3.3)	2.3(2.0, 2.5)	0.99	(1.5– 4.2)
T-CHOL /HDL ratio	2.5(2.0, 3.8)	2.9(2.1, 4.8)	0.07	3.5(2.0, 4.8)	3.4(3.2, 3.6)	0.61	<4
TG (mmol/L)	0.7(0.4, 1.5)	0.7(0.5, 1.4)	0.76	1.1(0.8, 1.3)	0.7(0.7, 0.7)	0.73	<1.7
FFA (mmol/L)	0.6(0.3, 1.1)	0.8(0.3, 1.6)	0.12	0.4(0.2, 0.8)	0.5(0.4, 0.6)	0.95	(0.4 to 0.8)
Adipokines							
Leptin (ng/ml)	4.4(1.1, 55.0)	2.4(0.9, 41.7)	0.24 0.45	11.9(2.1, 37.4)	17.4(10.0, 24.7)	0.88	
Leptin/FM	1.1(0.5, 5.2)	0.7(0.4, 7.4)	0.37	0.6(0.2, 1.3)	0.9(0.8, 1.0)	0.40	
Adiponectin (ng/l)	10.9 (5.9, 29.9)	15.5 (8.0, 20.6)	0.49	6.7 (1.7, 20.1)	9.7 (6.2, 13.2)	0.88	
Adiponectin/FM	2.5 (0.4, 14.7)	4.2(1.2, 10.8)	0.24	0.3(0.1, 1.4)	0.6(0.3, 1.0)	0.61	
Resistin (ng/ml)	3.9 (1.9, 7.0)	4.1(2.4, 12.4)	0.18	4.0(2.9, 22.3)	7.6(6.8, 8.5)	0.18	
Resistin/FM	0.9 (0.1, 3.2)	1.6(0.3, 3.0)		0.3(0.2, 0.8)	0.5(0.3, 0.7)	0.24	
Glucose homeostasis							
F-Glucose (mmol/L)	4.6 (3.5, 5.3)	4.4(3.7, 5.2)	0.41	4.2(4.0, 4.5)	4.5(4.4, 4.6)	0.17	(3.5-5.5)
F-Insulin (uIU/ml)	8.3 (4.2, 57.8)	11.1(1.6, 103.8)	0.73	12.5(10.0, 45.8)	20.3(7.5, 33.1)	0.84	<20*
HOMA-IR	1.8 (0.9, 13.4)	2.2(0.3, 19.4)	0.68	2.3(1.9, 8.5)	4.0(1.5, 6.5)	0.56	<4.5*

T-CHOL: total -cholesterol LDL: Low Density Lipoprotein Cholesterol; HDL: High Density Lipoprotein Cholesterol; TG: triglyceride; FFA: free fatty acid; F: fasting; HOMA-IR: Homeostatic model assessment; FM: fat mass.
* Normal ranges of fasting insulin and HOMA-IR were according age- and sex-specific paediatric reference interval as indicated in references (453,454).

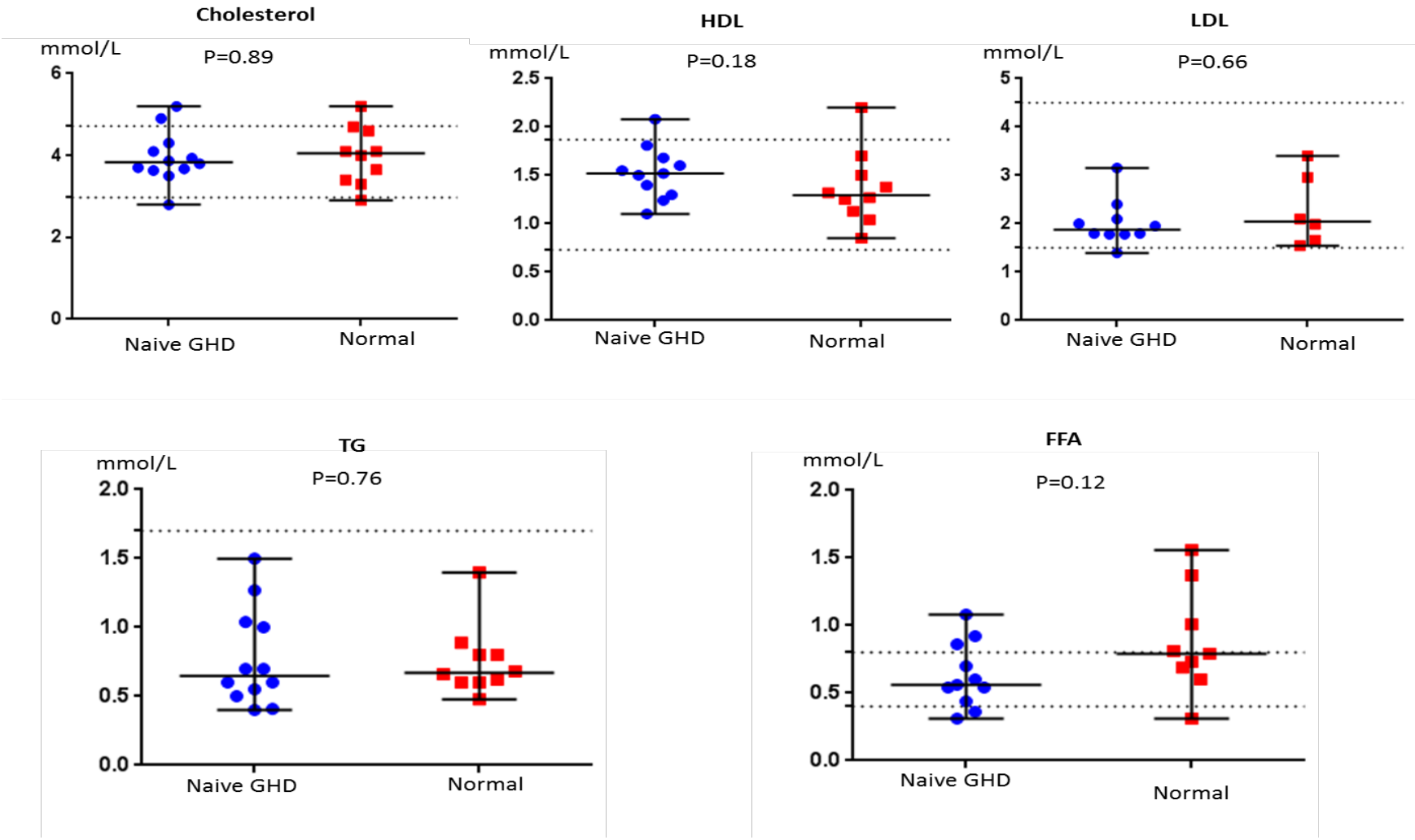

Figure 6-1 Individual data of lipid profiles in the first time assessment group.
LDL: Low Density Lipoprotein Cholesterol; HDL: High Density Lipoprotein Cholesterol; TG: triglyceride; FFA: free fatty acid

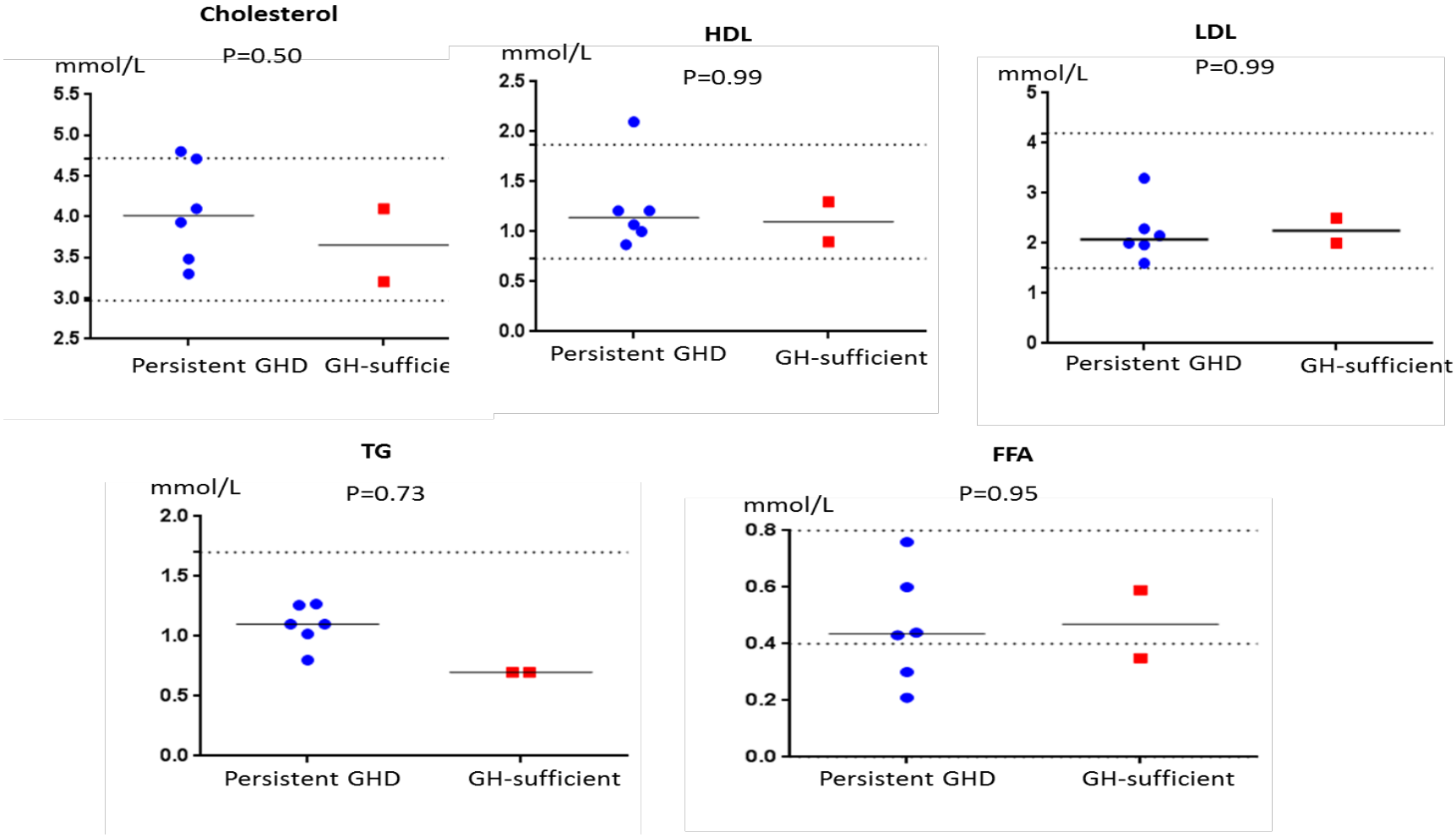

Figure 6-2 Individual data of lipid profiles in the retesting group.
HDL: High Density Lipoprotein Cholesterol; TG: triglyceride; FFA: free fatty acid.

6.5.3 Adipokines

From Table 6-2 and Figure 6-3, no differences were found in either absolute or fat mass (FM) adjusted values of serum concentrations of adipokines (leptin- adiponectin- resistin) between naive GHD and normal children in the first time assessment; even when obese children were excluded. However, the median of leptin/FM ratio was slightly higher in naive GHD compared to normal children [1.1 (0.5, 5.2) naive GHD vs. 0.7 (0.4, 7.4) normal] although not statistically significant (p= 0.42). The median ratios of adiponectin/FM and resistin/FM also tended to be lower in naive GHD than normal but insignificantly (2.5 vs. 4.2 and 0.9 vs. 1.6, respectively).

Absolute and FM adjusted adipokine levels in the retesting group were similar between those with persistent GHD and GH sufficient. However, those with persistent GHD tended to have lower leptin, adiponectin and resistin levels compared to those who were GH sufficient (Figure 6-4).

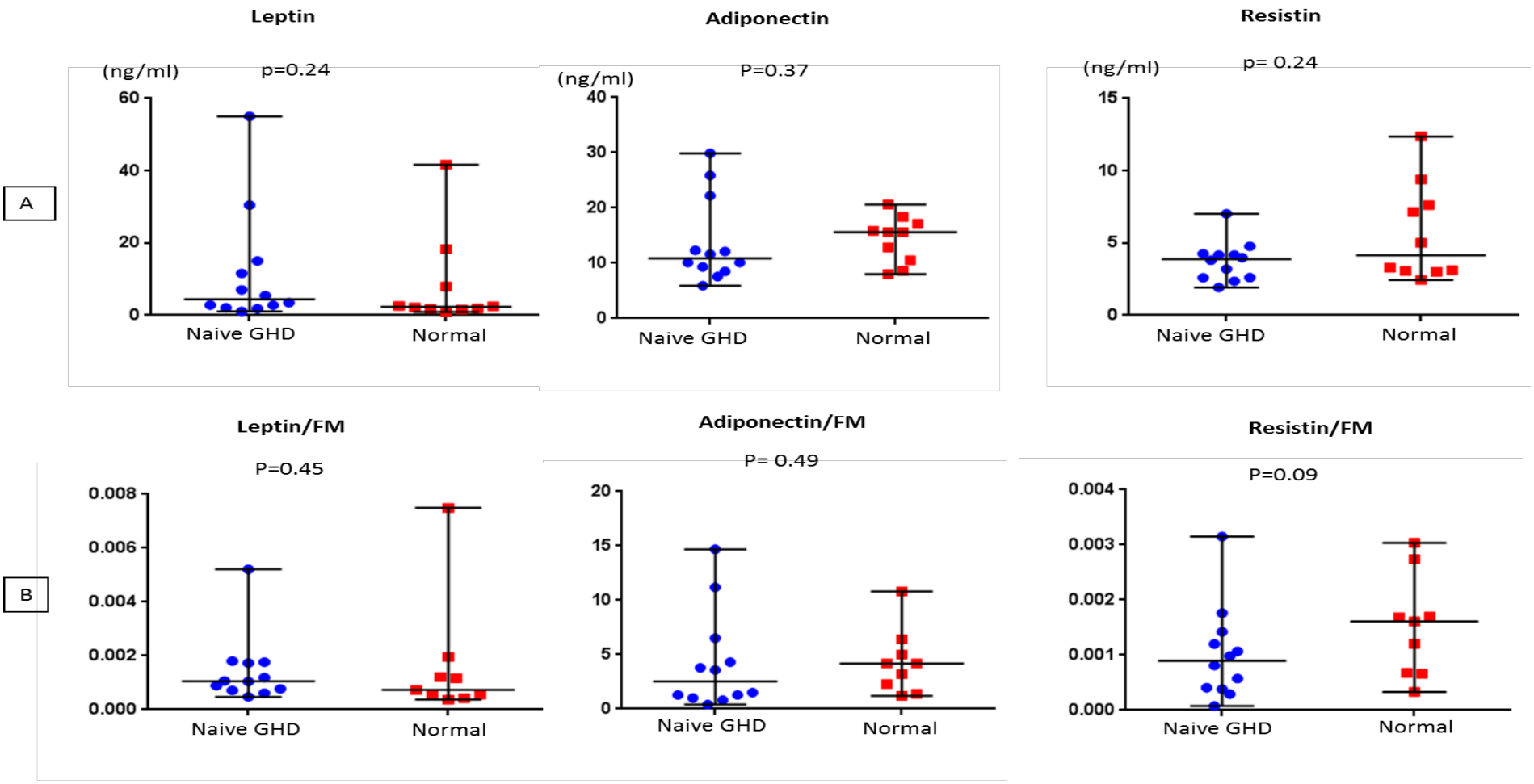

Figure 6-3 Individual data of adipokines levels in the first time assessment group.
(A absolute values - B adjusted to fat mass)

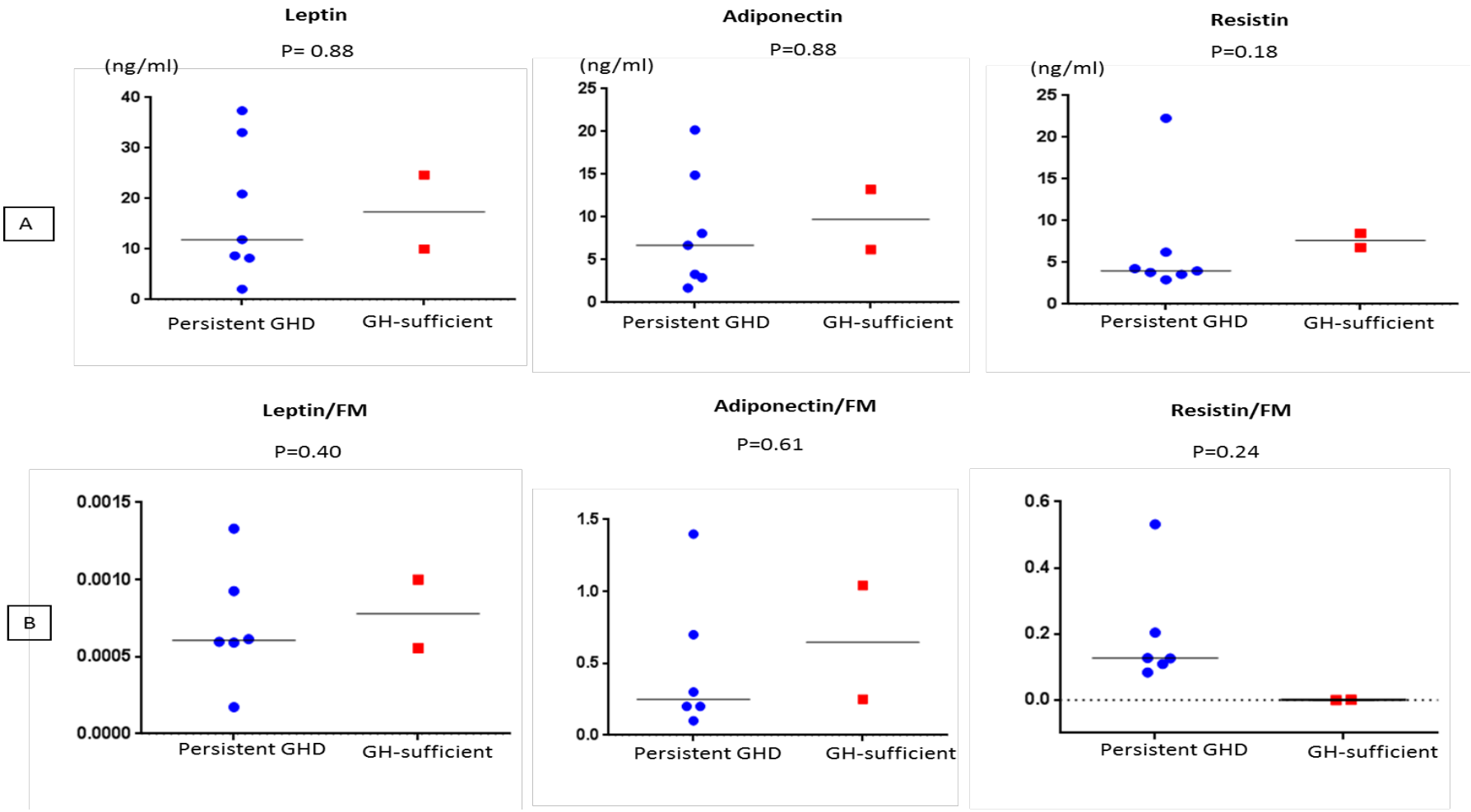

Figure 6-4 Individual data of adipokines levels in the retesting group.
(A absolute values - B adjusted to fat mass)

6.5.4 Glucose Homeostasis

Individual data of glucose homeostasis parameters in the first time assessment and the retesting groups are illustrated in Figure 6-5. In the first time assessment group, naive GHD tended to be more insulin sensitive with a lower HOMA-IR index compared to normal [1.8 (0.9-13.4) vs. 2.2 (0.3-19.4), respectively, p=0.68].

Five children in the first time assessment group had a HOMA-IR index at more than 4.5 (three naïve GHD, two normal), Table 6-3.

Table 6-3 Individual data of the first time assessment subjects with HOMA-IR > 4.5.
* BMI>+2 SDS (obesity)

	Age (yr)	sex	Tanner stage	GH-peak (µg/l)	GHD Aetiology and/or other morbidity	BMI	BMI SDS	F-Glucose	F-insulin	HOMA-IR
1	15.1	M	II	0.9	Idiopathic GHD	31.8	2.9*	5.2	57.8	13.4
2	14.9	M	III	0.7	Empty sella-GHD	18.6	-0.3	4.5	25.0	5.0
3	12.9	F	II	0.7	Idiopathic GHD	32.3	3.0*	5.1	25.2	5.7
4	14.2	F	II	18.6	Mitochondrial disease with primary ovarian failure	24.7	1.6	4.0	49.3	8.7
5	13.7	F	III	8.5	Autoimmune polyendocrinopathy ectodermal dystrophy	17.7	-0.7	4.2	103.8	19.4

HOMA-IR index was not different in those with reconfirmed persistent GHD in the retesting group compared to those with GH sufficiency (2.3 (1.9-8.5) persistent GHD, 4.0(1.5-6.5) GH sufficient, P=0.56), Figure 6-5. Two of those who were confirmed with persistent GHD and one who was GH sufficient among the retesting group have HOMA-IR >4.5 (8.5, 6.6, and 6.5 respectively) as shown in Table 6-4.

Table 6-4 Individual data of the retesting subjects with HOMA-IR >4.5

	Age (yr)	Sex	GH-peak (µg/l)	Aetiology and other morbidity	BMI	BMI SDS	F-Glucose	F-Insulin	HOMA-IR
1	15.1	F	3.8	IGHD (Idiopathic), Previous Celiac disease	28.0	2.0	4.2	45.8	8.5
2	14.9	M	3.2	MPHD (Oncology)	25.7	1.7	4.5	32.8	6.6
3	12.9	F	10.2	IGHD (Ectopic posterior pituitary)	24.1	1.1	4.4	33.1	6.5

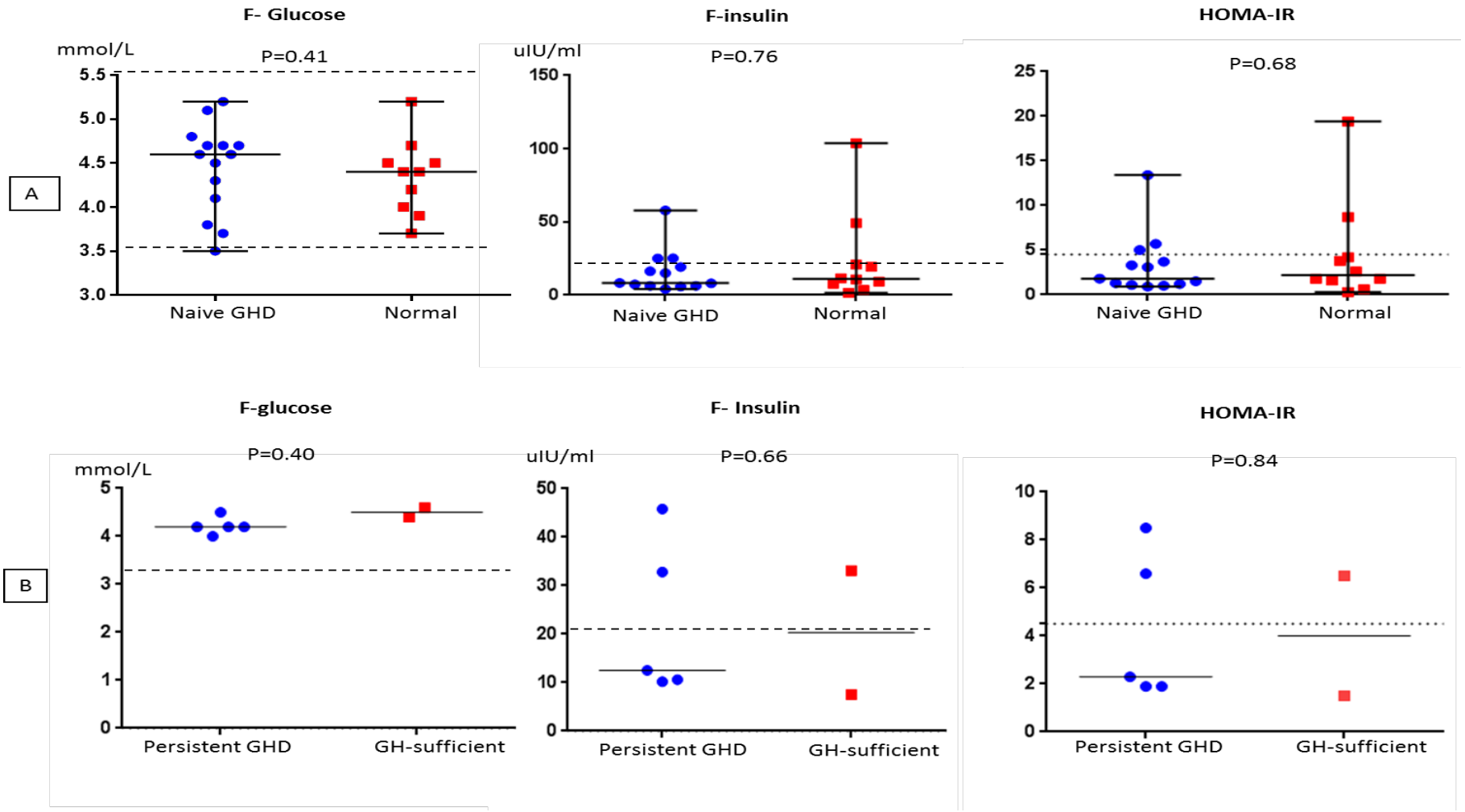

Figure 6-5 Individual data of glucose homeostasis in the first time assessment (A) and the retesting groups (B).
*Cut-off lines for fasting insulin (20) and HOMA-IR (≥4.5) were according to Viner et al 2012 (441)

6.5.5 Relation between body metabolic parameters and bone parameters

No associations were found between lipid profiles levels and any variables in our first time assessment group.

In the retesting group, we found total cholesterol levels at time of retesting were correlated positively with the age of starting rhGH treatment at childhood ($r=0.89$, $p=0.01$), but negatively with the duration of rhGH replacement during childhood: ($r=-0.95$, $p<0.01$) as seen in Figures 6-6, 6-7. However, the duration from stopping rhGH treatment to retesting was not associated with any adverse metabolic parameters in our cohort.

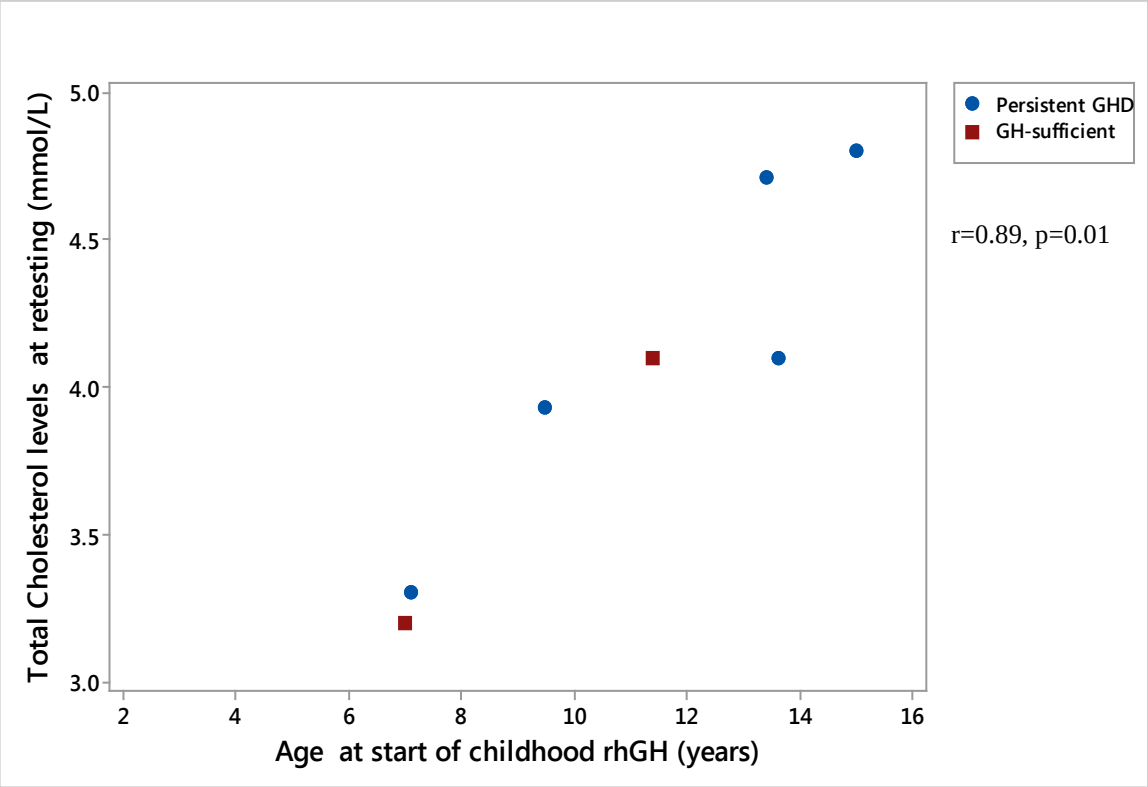

Figure 6-6 Scatterplots showing correlations between total cholesterol levels at retesting and age of starting childhood rhGH

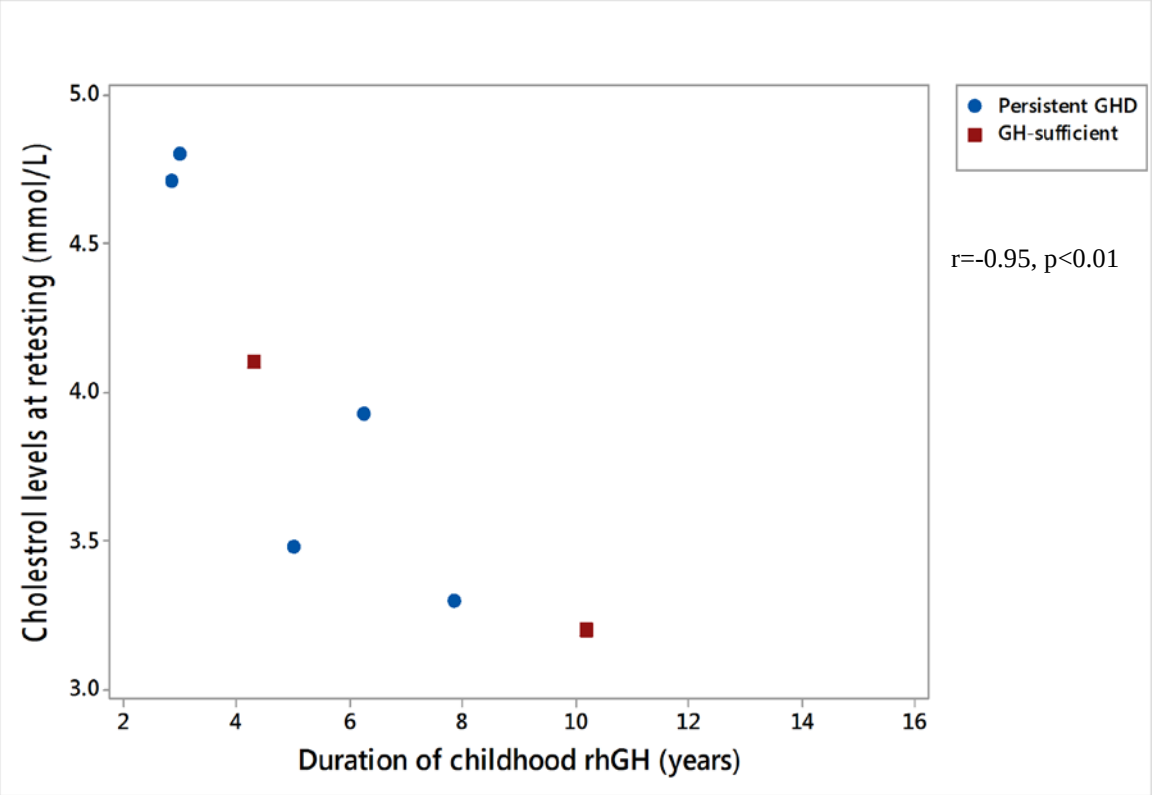

Figure 6-7 Scatterplots showing correlations between total cholesterol levels at retesting and duration of childhood rhGH

Table 6-5 reports Spearman correlations between adipokines, bone metabolism and body composition in the two groups.

Leptin was found to be positively and strongly correlated with BMI and FM in both first time assessment and retesting groups ($p<0.001$). No correlation between adiponectin and resistin with any other metabolic parameters in either group was observed even when adjusted for fat mass. In the retesting group age at childhood diagnosis correlated positively with leptin levels at retesting ($r=0.89$, $p<0.01$).

Leptin levels were also positively correlated with osteocalcin (OC) in the first time assessment group ($r=0.51$, $p=0.01$) but inversely with OC and bone-specific alkaline phosphatase (BAP) in the retesting group ($r= -0.91$, $P<0.01$: $r=-0.81$, $P<0.01$, respectively), as illustrated in Figures 6-8, 6-9.

Table 6-5 Spearman correction between adipokines and other metabolic and clinical data in the first time assessment and the retesting groups

	First time assessment N=25						Retesting n=9					
	Leptin		Adiponectin		Resistin		Leptin		Adiponectin		Resistin	
	r value	P value	r value	P value	r value	P value	r value	P value	r value	P value	r value	P value
Age (yr)	0.28	0.19	-0.40	0.06	0.07	0.73	0.00	0.90	0.26	0.48	0.33	0.38
BMI	0.55	<**0.01**	-0.33	0.13	-0.16	0.45	0.71	**0.04**	0.57	0.91	0.04	0.91
GH-peak (μg/l)	-0.23	0.29	0.18	0.41	0.31	0.14	0.26	0.53	0.47	0.23	0.64	0.08
IGF-1 levels (ng/ml)	0.18	0.42	-0.16	0.48	0.03	0.89	-0.30	0.43	-0.40	0.28	-0.08	0.83
Cholesterol(mmol/L)	0.15	0.49	-0.20	0.36	-0.27	0.22	0.39	0.33	-0.04	0.91	-0.28	0.49
TG(mmol/L)	0.47	**0.02**	-0.08	0.71	0.24	0.26	0.06	0.88	-0.53	0.17	-0.57	0.13
FFA(mmol/L)	0.40	0.06	0.22	0.35	0.03	0.90	-0.26	0.53	0.31	0.45	-0.11	0.77
F-Glucose(mmol/L)	0.41	0.06	-0.17	0.43	0.02	0.92	-0.22	0.63	0.00	0.90	0.03	0.93
F-Insulin(uIU/ml)	0.52	**0.01***	-0.20	0.35	0.15	0.48	0.64	0.11	-0.03	0.93	0.21	0.64
HOMA-IR	0.56	<**0.01***	-0.24	0.28	0.19	0.39	0.42	0.33	-0.17	0.70	0.00	0.97
BAL (μg/l)	0.37	0.08	-0.26	0.23	0.19	0.37	-0.81	<**0.01***	-0.18	0.63	-0.61	0.07
OC (ng/ml)	0.51	**0.01***	-0.35	0.10	0.32	0.14	-0.91	<**0.01***	-0.35	0.35	-0.60	0.08
CTX (ng/ml)	0.14	0.51	-0.10	0.63	-0.13	0.54	0.36	0.33	-0.35	0.35	0.23	0.54
LM (kg)	0.21	0.35	-0.46	0.32	0.07	0.97	0.00	0.99	-0.66	0.09	-0.09	0.82
FM (kg)	0.82	<**0.01**	-0.30	0.18	0.05	0.80	0.92	<**0.01**	0.28	0.49	0.28	0.49
LM/FM ratio	-0.82	<**0.01**	0.01	0.96	0.06	0.78	-0.85	<**0.01**	-0.66	0.07	-0.38	0.35
Age of childhood diagnosis(yr)	-	-	-	-	-	-	0.89	<**0.01***	0.07	0.87	-0.14	0.76
Duration of childhood rhGH (yr)	-	-	-	-	-	-	-0.51	0.15	-0.08	0.83	0.23	0.54
Duration of stopping rhGH(yr)	-	-	-	-	-	-	0.21	0.57	-0.07	0.84	-0.00	0.98

BMI: body mass index; IGF-1 insulin growth factor -1; TG: triglyceride; BAL; bone-specific alkaline phosphatase; OC: Osteocalcin; CTX: cross linked C-telopeptide of type I collagen; LM: lean mass; FM: fat mass; * Not significant when adjusted for FM

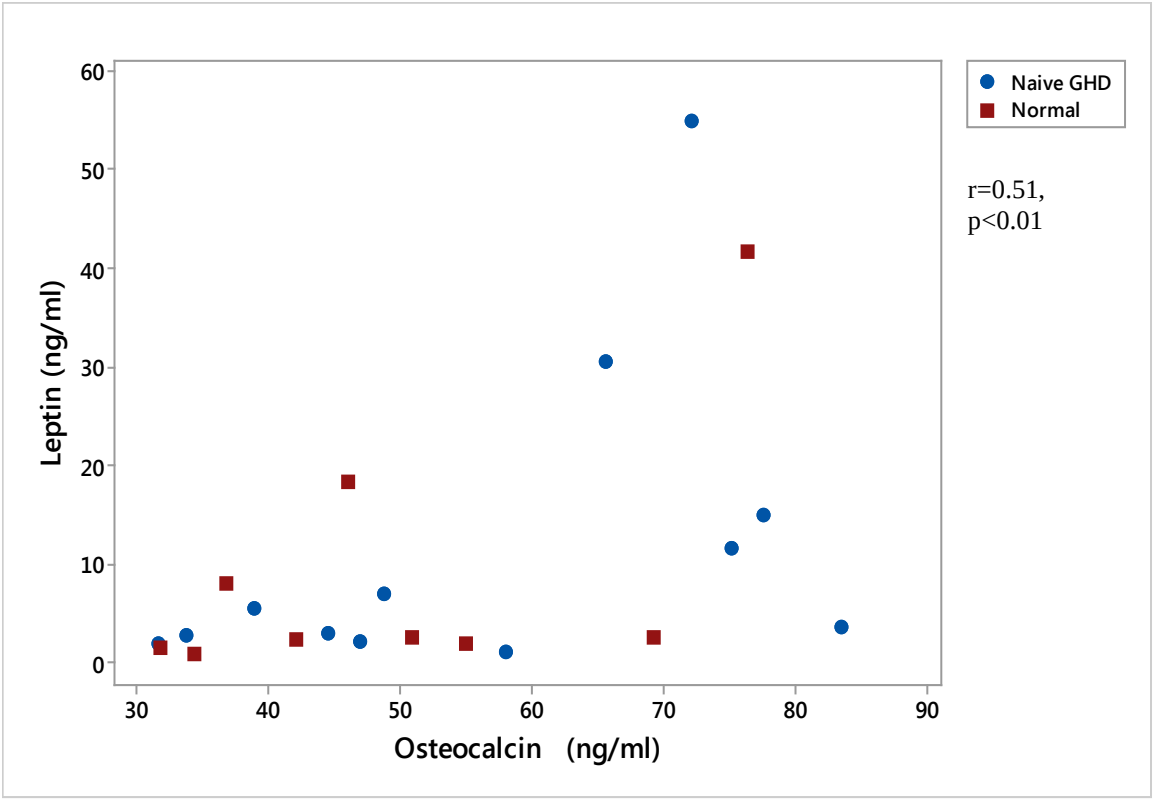

Figure 6-8 Scatterplots showing correlations between leptin and osteocalcin in the first time assessment group

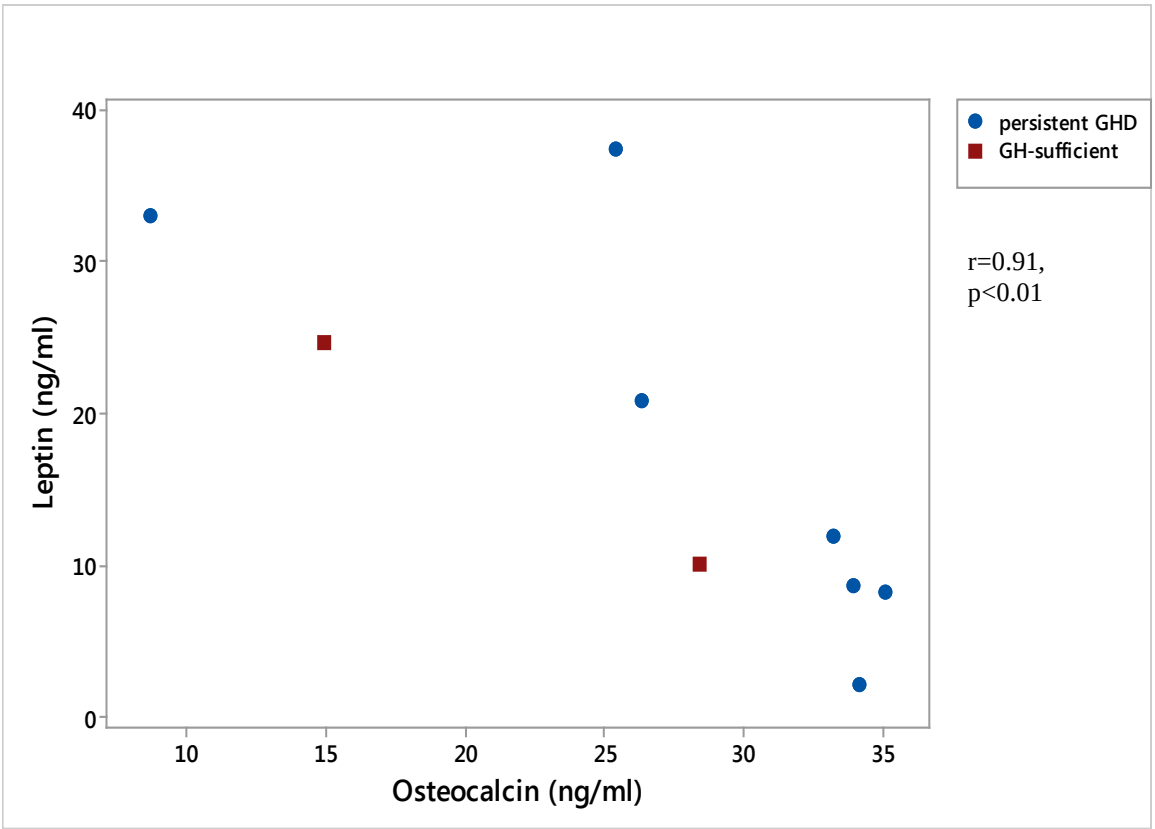

Figure 6-9 Scatterplots showing correlations between leptin and osteocalcin in the retesting group

Table 6-6 summarised Spearman correlations between glucose homeostasis parameters and clinical, metabolic data in both studied groups. We found a statistically significant direct correlation between HOMA-IR and FM in the first time assessment and the retesting group ($r=0.70$, $P<0.01$, $r=0.88$, $P=0.01$, respectively), HOMA-IR with LM ($r=0.78$, $p<0.01$) and LM/FM ratio ($r= -0.45$, $p= 0.03$) (Figure 6-10) only in the first time assessment. There were also correlations between fasting insulin levels and HOMA-IR with both BAP and OC in the first time assessment group (F-insulin($r=0.55$, $P=0.04$; $r=0.80$, $P<0.01$, respectively): HOMA-IR, $r=0.77$, $p=0.03$; $r=0.56$, $p<0.01$, respectively) but these observations did not reach statistical significance in the retesting group.

Table 6-6 Spearman correlations between glucose homeostasis parameters and clinical, metabolic in the first time assessment and the retesting groups

	First time assessment (n=25)						Retesting group (n=9)					
	F-Insulin		F-Glucose		HOMA-IR		F-Insulin		F-Glucose		HOMA-IR	
	r value	P value	r value	P value	r value	P value	r value	P value	r value	P value	r value	P value
Age (yr)	0.76	**<0.01**	0.16	0.44	0.85	**<0.01**	-0.14	0.76	-0.22	0.63	-0.39	0.38
BMI	0.71	**<0.01**	0.33	0.11	0.67	**0.01**	0.88	**0.01**	-0.27	0.60	0.94	**<0.01**
GH peak (µg/l)	0.00	0.97	-0.19	0.36	0.02	0.92	0.32	0.48	0.63	0.12	0.21	0.64
IGF-1 levels (ng/ml)	0.55	**<0.01**	0.11	0.60	0.76	<0.01	0.14	0.76	0.81	**0.02**	0.17	0.70
FM (kg)	0.75	**<0.01**	0.44	**0.03**	0.70	**<0.01**	0.94	**<0.01**	-0.09	0.86	0.88	**0.01**
LM (kg)	0.78	**<0.01**	0.17	0.42	0.76	**<0.01**	-0.39	0.43	0.25	0.62	0.37	0.46
LM/FM	-0.41	0.05	-0.43	0.04	-0.45	0.03	-0.60	-0.20	-0.03	0.95	0.39	0.39
Cholesterol (mmol/L)	0.22	0.33	0.26	0.24	0.24	0.28	0.36	0.42	-0.41	0.35	0.23	0.61
TG (mmol/L)	0.49	**0.02**	0.10	0.64	0.57	**0.01**	0.30	0.50	-0.34	0.45	0.45	0.30
FFA (mmol/L)	-0.33	0.16	-0.42	0.05	-0.35	0.13	-0.67	0.09	-0.37	0.41	-0.28	0.23
BAL (µg/l)	0.52	**0.01**	0.22	0.31	0.50	**0.01**	-0.35	0.43	0.44	0.31	-0.17	0.70
OC (ng/ml)	0.52	**0.01**	0.21	0.31	0.55	**<0.01**	-0.32	0.48	0.18	0.69	0.00	0.98
CTX (ng/ml)	0.18	0.41	0.34	0.10	0.17	0.45	0.67	0.09	-0.48	0.27	0.39	0.38
Age of childhood diagnosis (yr)	-	-	-	-	-	-	0.70	0.18	0.56	0.32	0.60	0.28
Duration of childhood rhGH							-0.46	0.29	0.18	0.69	-0.39	0.38
Duration of stopping rhGH(yr)							0.34	0.45	-0.35	0.43	-0.01	0.96

BMI: body mass index; IGF-1 insulin growth factor -1; TG: triglyceride; BAL; bone-specific alkaline phosphatase; OC: Osteocalcin; CTX: cross linked C-telopeptide of type I collagen; LM: lean mass; FM: fat mass

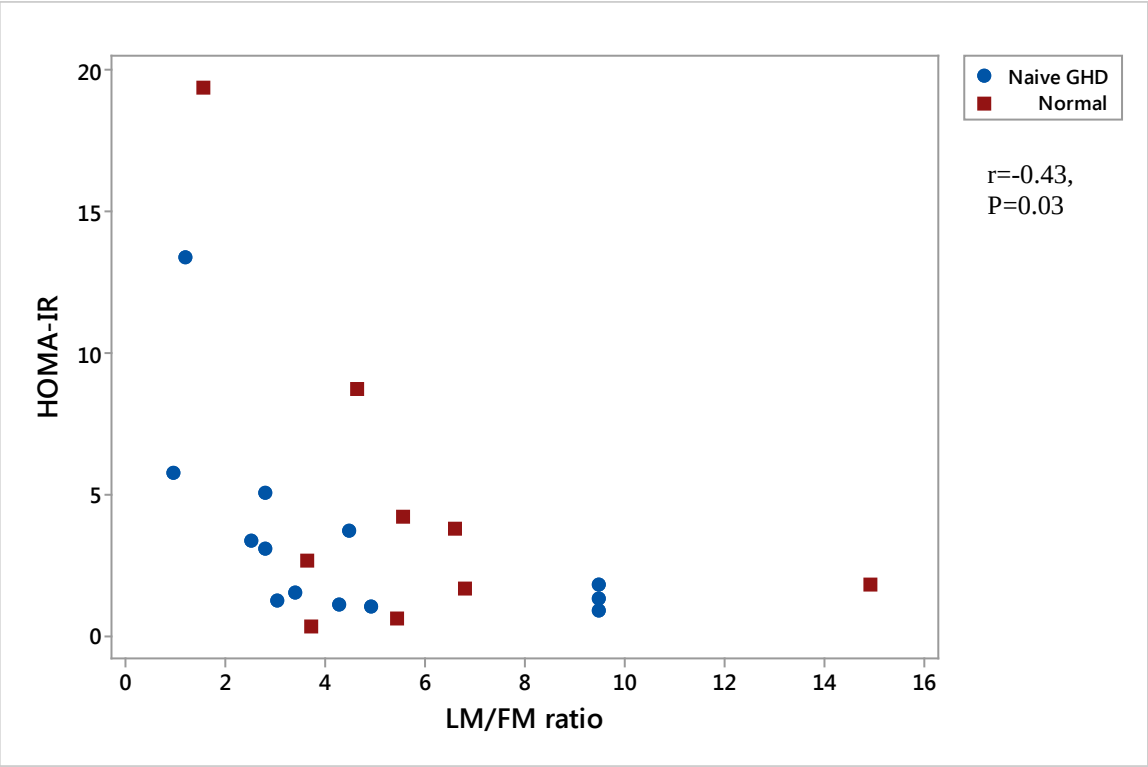

Figure 6-10 Scatterplots showing correlations between HOMA-IR and the ratio of lean mass to fat mass in the first time assessment group.

6.6 Discussion

The present study demonstrates no differences in lipids, adipokines and glucose homeostasis parameters in patients with CO-GHD and those with normal GH levels at time of initial evaluation and retesting after withdrawal of childhood rhGH at final height, with the majority of these parameters within the normal range.

Data in the literature regarding lipid profiles in CO-GHD at these two points in time are inconsistent. Studies found similar levels of unfavourable lipid profiles in untreated children with GHD at time of diagnosis compared to short stature controls (459) or slightly increased in GHD (440,441,450,451). Adolescents with CO-GHD are also reported to have unfavourable alterations in lipid profiles after discontinuation of rhGH treatment at final height as early as 6 months and up to two years in some studies (236,245,262,442,460), but not all (229,232,245,365).

In this study, we tried to identify factors related to CO-GHD that may be associated with alterations in lipids profiles after withdrawal of rhGH at final height. The novel finding of our analysis is that those who were older when first starting childhood rhGH and had a shorter duration of replacement before final height were more likely to have higher total cholesterol levels during transition. Although a similar observation was previously reported (264), our findings speculate delay in starting rhGH in these particular subjects results in altered lipid profiles, with potentially increased cardiovascular risk in the future, whether they were classified as GH deficient or sufficient on retesting. Therefore, early detection and starting rhGH treatment in GHD is not only better for height outcome, but also better for long-term metabolic and cardiovascular risks. It was reported previously that longer duration of discontinuation rhGH aggravated lipid profiles (264). From our data, however, it could be assumed that lipid alterations observed at the time of discontinuation of rhGH may be explained in part to the short-term effects of rhGH therapy and could not be considered independently of duration of treatment during childhood (269). This assumption was supported by evidence that emerged from studies of adults with GHD which revealed that only long duration of rhGH therapy (5-10 years) improved lipid profiles in adults with GHD (443,461). Further studies are needed to confirm this assumption in CO-GHD.

With regards to glucose homeostasis aspects, some studies reported that the subjects with CO-GHD were more insulin sensitive at time of initial diagnosis (444,462) and after withdrawal of rhGH at final height (244,245). Our data, however, did not reveal any significant differences between GHD and those with normal GH levels, either in insulin, glucose or HOMA-IR index, neither prior to starting childhood rhGH treatment or after discontinuation of rhGH at final height. This finding is consistent with previous studies in which no major differences have been found between GHD and non-GHD controls either at baseline or following rhGH therapy (440,441,450,463). In this study, HOMA-IR was correlated positively with IGF-1 but negatively with LM/FM ratio. From this finding and consistent with the existing evidence, it seems that glycometabolic parameters in subjects with CO-GHD are more likely to be related not only to the GH/IGF-1axis, but also to the

alterations in the lean to fat mass ratio. Considering the cut off guidance of Obesity Services for Children and Adolescents (OSCA) in the UK, a few of our subjects showed hyperinsulinemia and had a HOMA-IR > 4.5 which could be attributed to either higher BMI or other existing conditions. Also, these alterations may be related to temporary insulin resistance during the early stages of puberty in some of our cohort (464).

Our results have also indicated that adipokines (leptin, adiponectin and resistin) were comparable in GHD subjects and those with normal GH levels at time of diagnosis and retesting even when the influence of FM was eliminated. Conflicting data are available in the literature on adipokines levels in GHD patients. Similar to the present results, leptin, adiponectin and resistin levels were generally normal and comparable between children with GHD and healthy controls at time of initial diagnosis (440) but higher leptin and adiponectin were reported elsewhere (451). At present, there are limited data with regards to adipokine levels following discontinuation of rhGH at final height. A study found lower adiponectin levels in untreated GHD adolescents when compared to both treated GHD subjects and healthy controls (452). Similar to the literature, a strong correlation between leptin/ adiponectin and parameters of body composition (BMI-FM) was observed in our cohort (451,452). While a definite role of resistin in human metabolism has yet to be established (449), our study has shown no significant relationship between resistin with bone or body composition in both studied groups.

Although adipokines, especially leptin, have been extensively studied in recent years, the relationship between adipokines and bone turnover markers is not completely understood. In vitro studies suggested that leptin stimulates bone formation possibly by acting directly on marrow stromal cells to enhance osteoblast and inhibit adipocyte differentiation (465) or indirectly through stimulating the sympathetic nervous system which then signals to increase osteoblasts activity and decrease osteocalcin activity (156). However, clinical studies are less consistent with regard to the relationship between leptin and bone biomarkers. Previous studies revealed a negative relationship between leptin and OC in normal weight (466) and obese children and adolescents (467). Our analysis demonstrated a different relationship among our studied groups: a positive correlation in the first time assessment group but negative in the retesting group. This finding suggests that the relationship between leptin and OC in our first time assessment could be influenced by active growth, particularly as OC concentrations are known to vary with age, gender, height, growth velocity and puberty (468). Although GH/IGF-1 may act as an important modulator in production of adipose derivative adipokines (152), we were unable to demonstrate a significant relationship between the levels of GH and IGF-1 and adipokines. This result supports the hypothesis that the influence of adipokines, in particular leptin, on bone metabolism might be independent of the effects of IGF-1 and GH (469).

By contrast, it has been shown that not only are leptin and metabolic profiles involved in regulation of bone metabolism and turnover, but also that vice versa the bone metabolism turnover influences the endocrine regulation of glucose and fat metabolism (156,470). It is well established that leptin is strongly positively associated with insulin resistance independent of age, gender and BMI (471),

whereas OC was assumed to increase insulin secretion and sensitivity (470). Our data, however, showed a positive relationship between serum insulin and HOMA-IR with both bone formation biomarkers (BAP and OC) and leptin levels in the first time assessment group, but this was not evident at final height in our retesting group.

We must take into account the small sample size of this study that limits the power to properly control for confounding variables and determine true associations. In the retesting group, only two were GH sufficient which makes drawing statistical conclusions difficult. However, our data has great validity as venous blood samples were collected in a fasting state and at the exact same time of day in each patient. Additionally, by adjusting adipokine levels for a direct measure of fat mass, we were able to examine differences among groups taking into account their actual adiposity.

6.7 Conclusion

In conclusion, metabolic and adiposity parameters were not altered in CO-GHD patients at the time of diagnosis prior to starting rhGH and re-testing after withdrawal of rhGH at final height. However, our findings suggested timing and duration of childhood treatment may influence adiposity parameters seen in adolescents with CO-GHD. Further research is needed for evaluating the potential role of adipokines in bone metabolism as well as to better understand how body adiposity contributes to bone and body metabolism in this population

CHAPTER 7

Quality of Life in Children and Adolescents with Childhood Onset Growth Hormone Deficiency

7.1 Abstract

Background: Health related quality of life (QoL) is increasingly considered as an important aspect in clinical practice to assess the impact of illness or health interventions on the individual's life. Childhood onset-growth hormone deficiency (CO-GHD) is reported to have an impact on QoL. So far, there is no conclusive evidence that deterioration in aspects of QoL are directly related to GHD.

Aim: To compare quality-of-life in a group of children and adolescents with CO-GHD at the time of initial evaluation and retesting at final height.

Patients and methods: In this cross-sectional study, subjects undergoing either GH stimulation tests for investigation for short stature (first time assessment group-total-18: 12 naive GHD, 6 normal: age range (5.6, 14.9)) or biochemical re-evaluation at final height after withdrawal of rhGH therapy (retesting group-total-8: 5 persistent GHD, 3 GH sufficient: age range (14.9, 20.2)) were asked to complete either 36-SF or AGHDA QoL questionnaires at the time of assessment of their GH axis.

Results: QoL was not significantly altered in children with naive GHD with total scores of SF-36 [93 (77, 96) naive GHD vs. 90 (84, 93) normal, P=0.56]. However, naive GHD subjects feel less energetic than normal controls (75 (65, 100) vs. 95 (65,100) respectively, p=0.04). Unexpectedly, subjects with normal GH levels in the first time assessment group scored lower in the subscale of emotional wellbeing compared to those with naive GHD (78 (55, 84) normal vs. 90 (68, 96) naive GHD, $p<0.001$). In the retesting group, those with persistent GHD scored better in the AGHDA questionnaire than GH sufficient individuals (6 points (2, 8) vs. 9 points (7, 17) respectively, but this was not significant ($p= 0.10$). Subscale analysis showed that GH sufficient subjects significantly lacked energy and complained of tiredness compared to those who were confirmed to have persistent GHD (1 point (0, 1) persistent GHD vs. 5 points (3, 6) GH sufficient, $p= 0.03$). There were no correlations between the levels of either stimulated GH peak or IGF-1 and total QoL scores in either group. However, IGF-1 levels at time of retesting correlated inversely with the subscale of memory and concentration in the AGHDA questionnaire.

Conclusion: Neither questionnaire found significant differences in total QoL scores between patients with CO-GHD and those who had normal GH levels at time of initial evaluation or retesting at final height. Further studies to validate QoL specific instruments in this population are needed with greater insight to elucidate factors that modify the relationship between GH status and QoL in children and adolescents

7.2 Introduction

Quality of life (QoL) has been defined broadly by the World Health Organization as 'the state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity' (472). The relationship between childhood onset growth hormone deficiency (CO-GHD) and QoL has not been clearly detailed. Emerging data suggests that QoL is impaired in short stature children and adolescents with CO-GHD or idiopathic SS (ISS) (473,474) and that recombinant human growth hormone (rhGH) treatment may improve QoL in those children (475-477). However, the evidence of improvements in QoL with rhGH particular during childhood is not still strong enough to be conclusive. Some studies suggested that the impact of rhGH on QoL relates to its influence on stature (478), whereas other data showed no evidence to support the link between height and QoL (479) and an increase in height does not guarantee an improvement in the quality of life (275,480-482).

Similarly, during transition from childhood to adulthood, studies suggest that adolescents with CO-GHD who were treated with rhGH and discontinued at final height have some psychological problems compared to healthy peers, which are reported to improve significantly on recommencing rhGH treatment (242). A study showed an inverse relationship between QoL and duration off GH therapy with a longer period off rhGH associated with a poorer QoL (264)-whereas re-instituting rhGH treatment has a significant positive change in health related QoL aspects (242,264). Inversely, other studies did not find any effect of either GHD or rhGH replacement on QoL in this particular population (232,247,274).

Perhaps it should be noted the fact that measuring QoL in children and adolescents with CO-GHD is challenging. One confounding factor in a such issue is the range of tools that have been used to measure QoL in relation to GHD including both generic and disease-specific questionnaires as summarised in Table 7-1 by Hull & Harvey 2003 (175). Unfortunately, the existing disease specific questionnaires were designed for adults with GHD with no available disease specific questionnaires for children with CO-GHD. However, a number of generic measures such as (KINDL, Pediatric Quality of Life Inventory (PedsQL), Child Health Questionnaire (CHQ)) have been developed to quantify QoL in children on self-reporting or on parental report and they have been applied to GHD children (481,482), but have not been well validated nor extensively used.

Giving that, it is imperative that QoL in CO- GHD children and adolescents be assessed separately from stature and prior to undergoing investigation and confirmation of the diagnosis of CO-GHD. The goal of this study therefore is to evaluate QoL of children and adolescents with CO-GHD at time of initial evaluation and retesting after withdrawal rhGH at final height.

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Table 7-1 Questionnaires tools measures used to assess quality of life in relation to GH

Generic Tools	Disease Specific Tools
AS – Apathy Evaluation Scale	
BDI – Beck Depression Inventory	QLS(M)-H – Questions on Life Satisfaction
BSI – Brief Symptom Inventory	Modules-Hypopituitarism
CIS – Clinical Interview Scale	DSQ – Disease Specific Questionnaire
CPRS – Comprehensive Psychological Rating Scale	AGHDA – Adult Growth Hormone
CHQ- Child Health Questionnaire	Deficiency Assessment
DSQ – Disease Specific Questionnaire	GHD-LFS – Modified Life Fulfillment Scale
GHQ – General Health Questionnaire	GHD-IS – Modified Impact Scale
GWBS – General Well-Being Schedule	GHDQ – Growth Hormone Deficiency
HDS - Hamilton Depression Scale	Questionnaire
HSCL – Hopkins Symptoms Check-List	
HADS – Hospital Anxiety and Depression Scale	
KSQ – Kellner Symptom Questionnaire	
KINDL	
LFS – Life Fulfilment Scale	
LSC – List of Somatic Complaints	
MADRS – Montgomery Asberg Depression Rating Scale	
MFQ – Mental Fatigue Questionnaire	
MFS – Mental Fatigue Scale	
MMPI-2 - Minnesota Multiphasic Personality Inventory-2	
MACL – Mood Adjective Check List	
NHP – Nottingham Health Profile	
PAS – Personality Assessment Schedule	
PedsQL- Pediatric Quality of Life Inventory	
POMS – Profile of Mood States	
PGWB – Psychological and General Well-Being Schedule	
SES – Self Esteem Scale	
SF-36 – Short Form 36	
SAS – Social Adjustment Scale	
SRS – Social Relationship Scale	
SCL-90 – Symptom Check-List-90	
SQ – Symptom Questionnaire	

(Adapted and developed from Hull & Harvey 2003(175))

7.3 Subjects and Methods

7.3.1 Patient characteristics

The original study design, inclusion and exclusion criteria have been described in chapter 5. Enrolment into the study occurs on a day when the patient attends for clinical investigations of GH axis. We analysed baseline data of 36 consecutively documented patients enrolled in the original study. In preliminary analyses, patients were classified into two age groups: first time assessment group and retesting group.

7.3.2 Quality of life assessment

In this study, QoL was evaluated using: short form-36 (SF-36) generic health survey for first time assessment group and Quality of Life Assessment in Growth Hormone Deficiency in Adults (AGHDA) for retesting group. After obtaining the informed consent, all study participants (or one of their parents) were asked to fill out one of QoL questionnaire form. Copies of the questionnaires can be found in the Appendix B and C.

7.3.2.1 Short Form-36 (SF-36)

SF-36 is a multi-item generic health survey intended to measure general health concepts not specific to any age, disease or treatment group. The SF-36 was first time published in 1992 (483) with the revised versions published in 2000 (484). The UK version was used in the present study. Although it is a generic measure, SF-36 measure has been shown to have high reliability, criterion validity and discriminant validity. It is easy to administer and does not require too much time and effort for its completion and has age-sex-based normal references for adolescents and adults (485,486), but not for children. SF-36 has been widely applied for children and adolescents with GHD (232,487,488). The SF-36 has 8 subscales with Likert scales of 2 to 6 response options that reproduce the two summary scores of the physical components scores (PCS) and the mental components scores (MCS) and an additional item on perceived change in health over the previous year**,** Figure 7-1. SF-36 subscale scores range from 0 to100, (higher scores indicating better functioning). Instruction of scoring and scales is available at link **http://www.rand.org/health/surveys_tools/mos/mos_core_36item.html**.
The participants' parent (or care giver) self-completed a questionnaire SF-36 in relation to their child at the time of investigation, children older than 8 to 15 years were asked with their parent's help to complete the questionnaire.

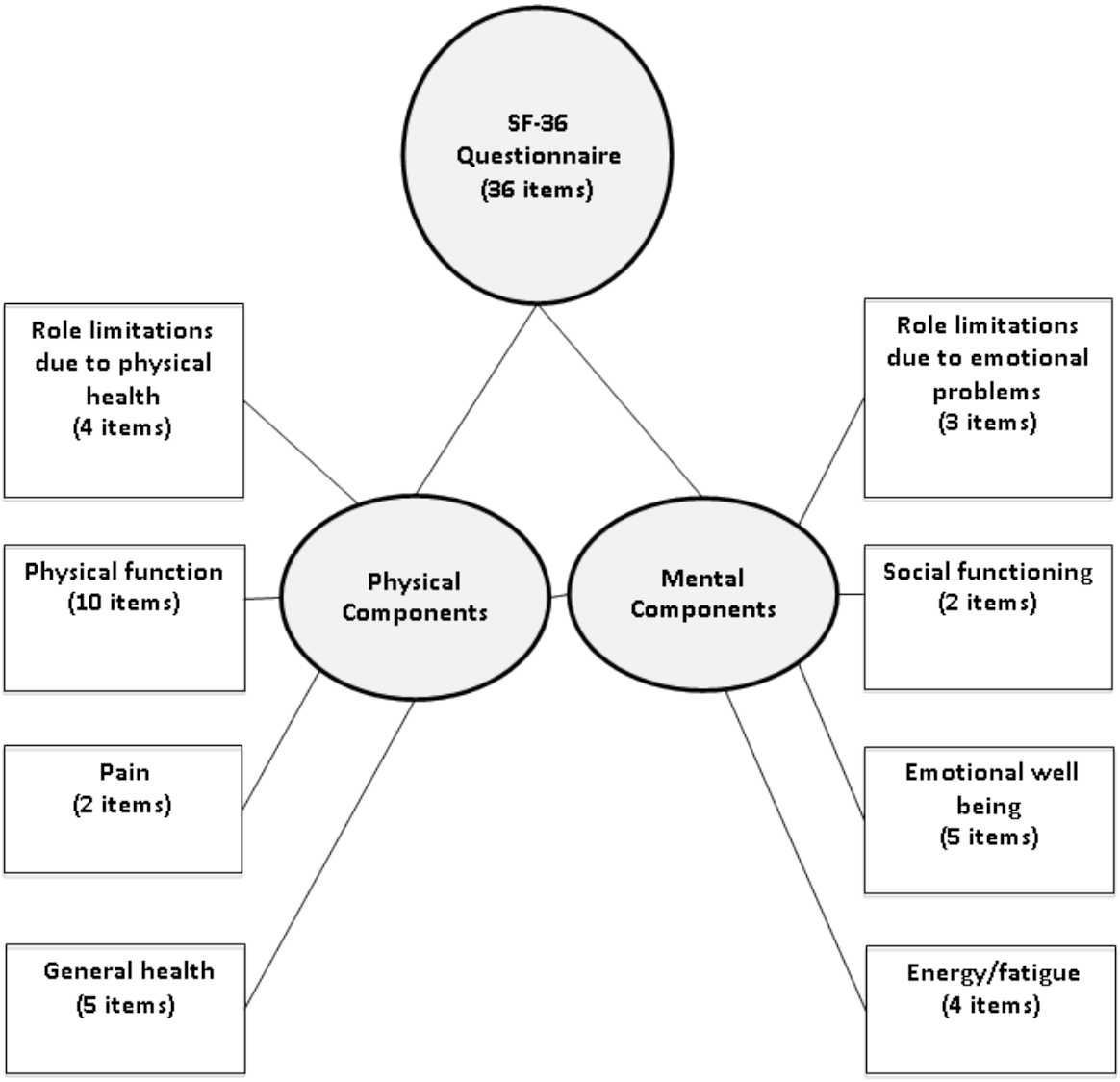

Figure 7-1 SF-36 Sub-scales measure physical and mental components of health

7.3.2.2 Quality of Life Assessment of Growth Hormone Deficiency in Adults (AGHDA)

AGHDA is a disease-specific, unidimensional, patient needs-based QoL questionnaire. This questionnaire had been validated in GHD patients from different countries and across languages. In the normal population, the mean values of total AGHDA scores range between 4 and 7 in various international studies, with higher scores indicating reduced QoL (489,490). A score of 11 or more on the QoL-AGHDA is one of the UK National Institute for Health and Clinical Excellence's requirements (NICE) for rhGH replacement therapy in adults (273). AGHDA has been used previously in assessment QoL of adolescents with CO-GHD (232,274). The 25 items in AGHDA clustered into five domains: memory and concentration (six questions), tiredness (seven questions), tenseness (three questions), social isolation (five questions) and self-confidence (four questions), Figure 7-2. All patients with CO-GHD have self-completed an AGHDA questionnaire at the time of retesting. The sum of 'yes' responses constitute a score, with a high score denoting a poor QoL.

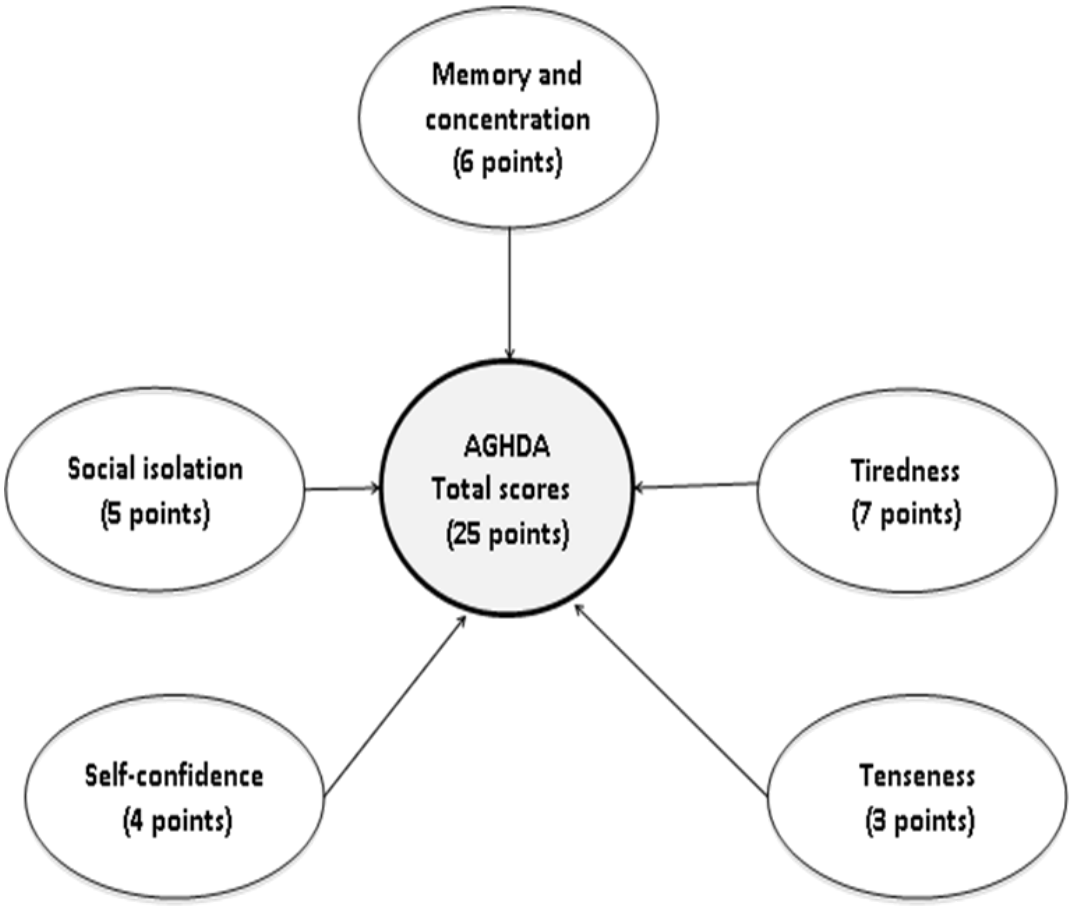

Figure 7-2 Quality of Life Assessment of Growth Hormone Deficiency in Adults (AGHDA)

7.4 Statistical Analyses

Statistical analysis was performed with Minitab17 (Minitab, Coventry, UK), with significance set at a level of < 0.05. Data were presented as median and ranges and inter-group differences were assessed using Mann-Whitney tests. The correlation between the variables was measured by Spearman rank test. All graphs were performed using GraphPad Prism 6 software (GraphPad Software, San Diego, CA, USA).

7.5 Results

7.5.1 Study population

Auxological and clinical characteristics of both groups were described in chapter 5.
For purposes of this study, we have excluded any patients who have another known underlying illness which could affect their physical or mental aspect and influence their overall score and those with missing data in both groups. Only 18 subjects within the first time assessment group and eight subjects of the retesting group were included in this study, Figure 7-3.
A total of 12 of the 18 subjects among the first time assessment group were confirmed to have isolated GHD (GH peak < 6.6 μg/l), in whom MRI was performed: six patients showed structural hypothalamic-pituitary abnormalities (n=2 ectopic pituitary, n= 1 arachnoid cyst, n=1 nerve gliomas, n=1 craniopharyngioma, n=1 empty sella syndrome), five patients showed normal MRI (idiopathic GHD), with no access to the MRI report in one patient. Of the remaining, 6 of the 18 had normal GH levels and were apparently healthy children without any known diseases or disorders.

The retesting group of adolescents with CO-GHD (n=8) were diagnosed at median age 9.5yrs(2.6, 12.0) and started treatment with rhGH during childhood at age 10.3 yrs(7.1, 13.6) with the median duration of treatment 5.6 yrs(4.1, 10.2). Four patients of the 8 had isolated GHD, three of them had idiopathic GHD and one had an ectopic posterior pituitary on MRI scan. The other four patients had multiple pituitary hormone deficiencies (MPHD) and received additional hormonal replacement therapy. Of those, one had hypoplastic pituitary on MRI scan, two had tumour related cranial irradiation and one with craniopharyngioma. All patients received appropriate replacement, where necessary, with glucocorticoids, thyroxine, sex steroids and desmopressin. All patients' ceased rhGH treatment at final height to re-evaluate their GH status at age 15.8yrs (14.4, 20.0) either by stimulation tests (n=6) and /or IGF-1 levels alone (n=2).
After retesting, 5/8 of them were reconfirmed to have persistent GHD (low GH peak <5 μg/l, and/or low IGF-1levels <-2 SD for age/sex references) and were eligible for adult rhGH therapy. Individual clinical characteristics data of our retesting group show in Table 7-2.

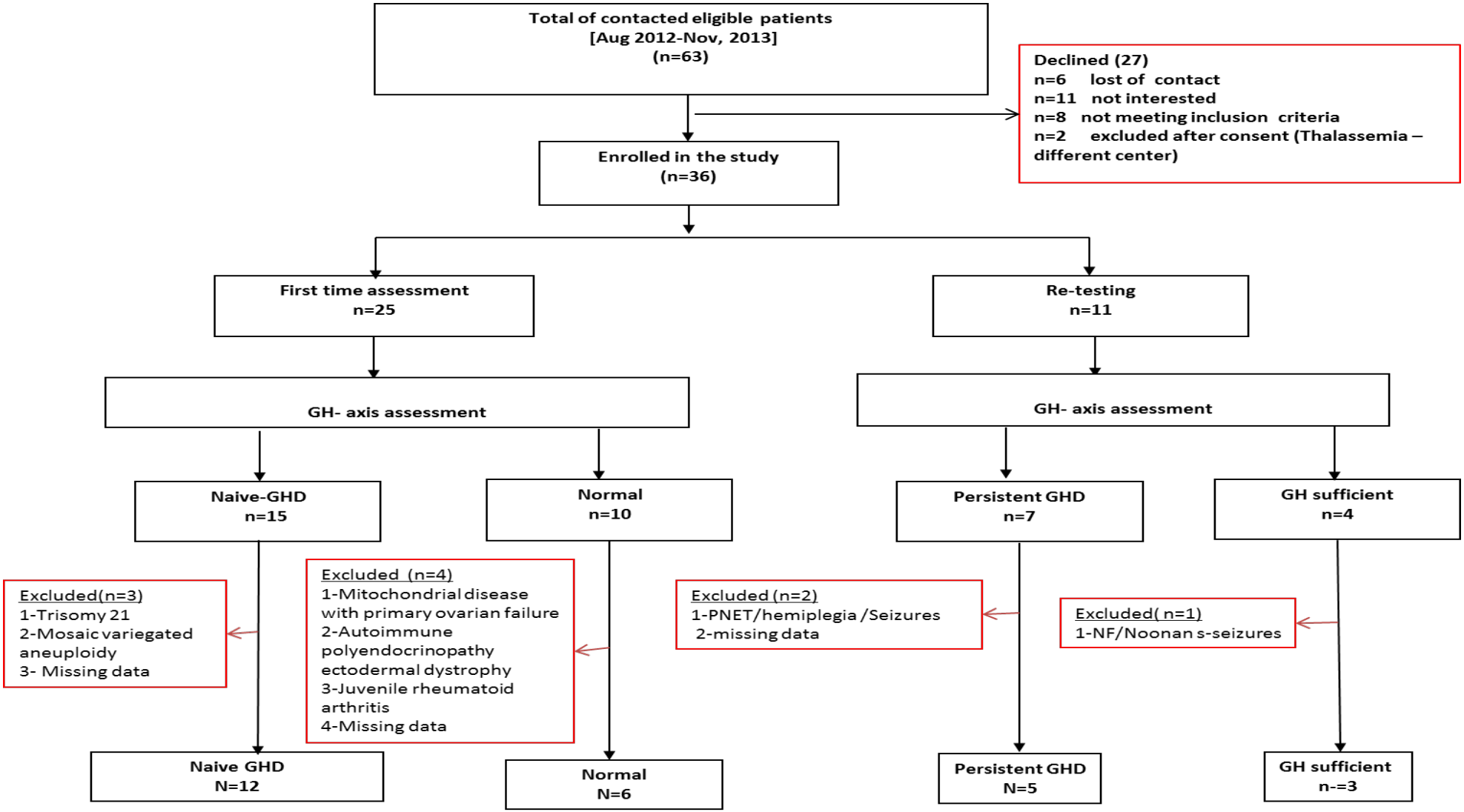

Figure 7-3 Flow diagram of study recruitment process

Table 7-2 Clinical characteristics of the retesting group

ID	Aetiology	Age (yrs)	Sex	Pituitary -MRI	Other pituitary hormones replacement	Duration of childhood rhGH (yrs)	Duration of off rhGH therapy (yrs)	Retesting GH peak (µg/l)	Retesting IGF-1 (mg/l)+
1	IGHD	16.6	F	Normal	-	5	0.6	3.8	90
2	MPHD (GHD-hypogonadism)	18.6	F	Hypo plastic pituitary	Ethnylestradiol	4.3	0.7	3.3	148
3	Craniopharyngioma-MPHD	14.9	F	Oncology	Hydrocortisone	4.1	0.5	-	45
4	Multisystem langerhans cell histiocytosis-MPHD	15.2	M	Oncology	Desmopressin	7.8	0.2	0.8	140
5	Large cell anaplastic lymphoma-MPHD	16.8	M	Oncology	Thyroxin-Testosterone	6.3	0.7	0.7	269
6	IGHD-22 deletion	17.0	F	Normal	-	10.2	0.3	6.4	205
7	IGHD-Obesity (BMI SDS=3.1)	20.4	M	Normal	-	8.0	0.7	-	137*
8	IGHD	16.3	F	Ectopic posterior pituitary	-	4.3	0.6	10	327

+ Normal IGF-1 levels (96-417 mg/l)

* This patient who had CO-GHD was re-tested by only checking IGF l level as he failed to attend stimulation tests three time and was lost to follow up , however, on clinical re-evaluation he was most like to be GH sufficient

The baseline relevant clinical and anthropometric data of both studied groups are presented in Table 7-3. There were no differences between the examined groups according to age, weight, height, and BMI. Stimulated GH peaks were significantly lower in naive GHD group than in normal (2.5 μg/l (0.7, 4.2) vs. 7.5 μg/l (6.7, 15.0), respectively, $p<0.001$), but did not reach significant levels between those who confirmed with persistent GHD and those who were GH sufficient in the retesting group (2.1 μg/l (0.3, 3.8) vs. 8.3 μg/l (6.4, 10.2), respectively, $p=0.05$). IGF-1 concentrations were not significantly different between studied groups.

There were no significant differences in duration of childhood rhGH treatment and duration off rhGH between those with persistent GHD and GH sufficient levels within the retesting group.

Table 7-3 Baseline characteristics of the first time assessment and the retesting groups

	Naive-GHD (n=12)	Normal (n=6)	P-value	Persistent GHD (n=5)	GH sufficient (n=3)	P-value
Male/Female	11/1	5/1	0.91	2/3	1/2	0.99
Age(yrs)	10.5(5.6, 14.9)	8.2(5.8, 12.1)	0.12	16.6(14.9, 18.6)	17.0(16.3, 20.4)	0.39
Height (cm)	128.9(97.7, 152.2)	114.0(96.1, 138.4)	0.10	158.0(152.7,179.7)	155.9(145.3, 166.4)	0.84
Height -SDS	-2.4(-3.4, 1.3)	-2.4(-3.9, 1.4)	0.96	-1.3(-1.9, 1.2)	-1.3(-3.0, 0.5)	0.84
Weight (kg)	26.4(15.0, 63.4)	20.9(13.3, 33.8)	0.26	55.6(45.6, 69.9)	52.2(37.6, 66.7)	0.88
Weight-SDS	-1.8(-3.5, 1.1)	-1.4(-3.9, -0.5)	0.86	0.3(-1.8, 1.4)	-1.1(-3.2, 1.1)	0.88
BMI (kg/m^2)	16.2(14.2, 11.3)	16.7(14.4, 17.6)	0.81	20.9(18.8, 28.0)	20.9(17.8, 24.1)	0.84
BMI-SDS	0.1 (-1.1, 3.9)	0.6(-1.2, 1.6)	0.77	0.4(-1.1, 2.0)	-0.2(-1.4, 1.1)	0.84
GH-peak(μg/l)	2.5(0.7, 4.2)	7.5(6.7, 15.0)	**<0.001**	2.1(0.3, 3.8)	8.3(6.4, 10.2)	0.19
IGF1 levels(mg/l)	52.5(14.0, 433)	76.0(28.0, 140.0)	0.87	141.0(45.0, 269.0)	205.0(117.0, 327.0)	0.37
IGF1 levels SDS	-3.4(-5.5, 0.3)	-2.1(-3.1, 0.9)	0.15	-3.4(<-5.0, 1.5)	-2.5(-3.7, -0.9)	0.37
Duration of childhood rhGH treatment(yr)	-	-	-	5.0(4.1, 7.8)	8.0(4.3, 10.2)	0.25
Duration of stopping rhGH (yr)	-	-	-	0.6(0.2, 0.7)	0.6(0.3, 0.7)	0.98

7.5.2 SF-36 QoL scores in the first time assessment group

Before diagnosis and commencing rhGH, a total QoL scores as measured by SF-36 of first time assessment group were comparable between those who were confirmed naive GHD and normal, Table 7-4. The medians of physical components scores (PCS) score and mental components scores (MCS) in the SF-36 were also not significantly different between groups, Figure 7-4. However, in the subscale analysis, the “energy/fatigue” dimension was significantly worse in naive GHD compared to normal [75 (65,100) naive GHD vs. 95 (65,100) normal, (P=0.04)], when those with normal GH levels scored significantly lower in emotional wellbeing subscale compared to naive GHD [90 (68, 96) naive GHD vs. 78 (55, 84) normal, $p<0.001$], Figure 7-5, 7-6.

Table 7-4 SF-36 questionnaire scores in the first time assessment group.
(Higher scores reflect better QoL)

Subscale	Naive-GHD (n=12)	Normal (n=6)	P-value
Total QoL	93(77, 96)	90(84, 93)	0.56
Physical component	91(76, 100)	91(78, 96)	0.86
Physical function	100(80, 100)	100(95, 100)	0.38
Role limitations due to physical health	100(25, 100)	100(75, 100)	0.82
pain	100(60, 100)	100(60, 100)	0.79
General health	89(50, 100)	66(60, 85)	0.20
Mental component	92(75, 96)	91(77, 96)	0.44
Role limitations due to emotional problems	100(100, 100)	100(75, 100)	NS
Energy/fatigue	75(65, 100)	95(65, 100)	0.04
Emotional well being	90(68, 96)	78(55, 84)	<0.001
Social functioning	100(63, 100)	100(80, 100)	0.66
Health change from one year ago	50(50, 100)	50(50, 50)	NS

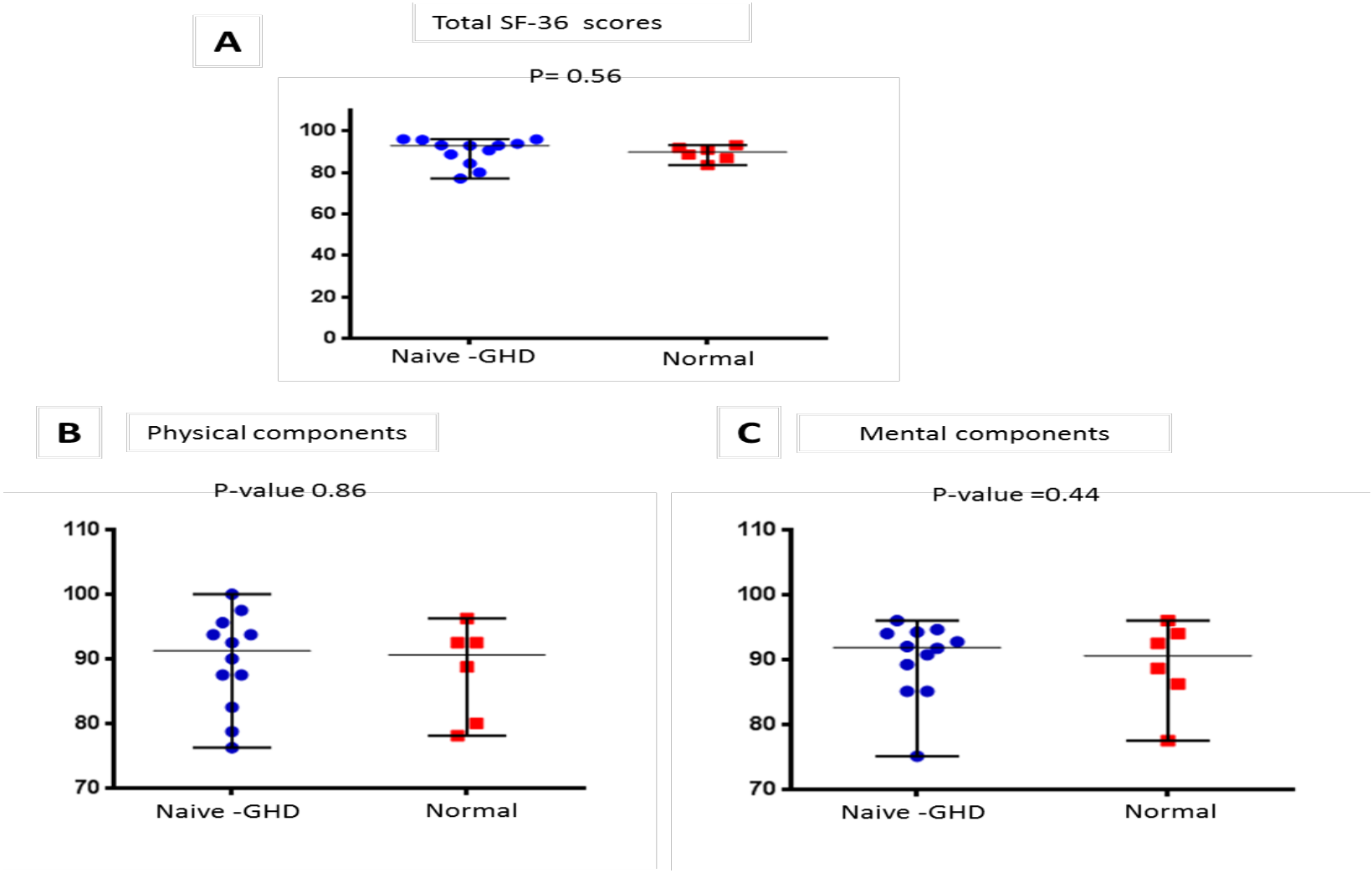

Figure 7-4 SF-36 QoL questionnaire scores in the first time assessment group.

A-Total scores, B-Physical components scores, C-Mental components scores

(Higher scores reflect better QoL)

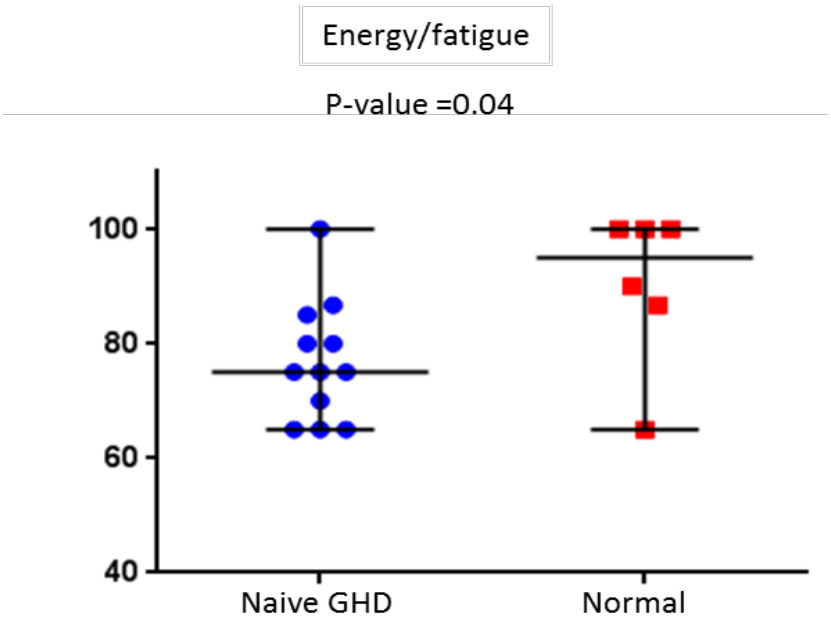

Figure 7-5 Individual data of energy and fatigue aspect of SF-36 QoL in the first time assessment
(Higher scores reflect better QoL)

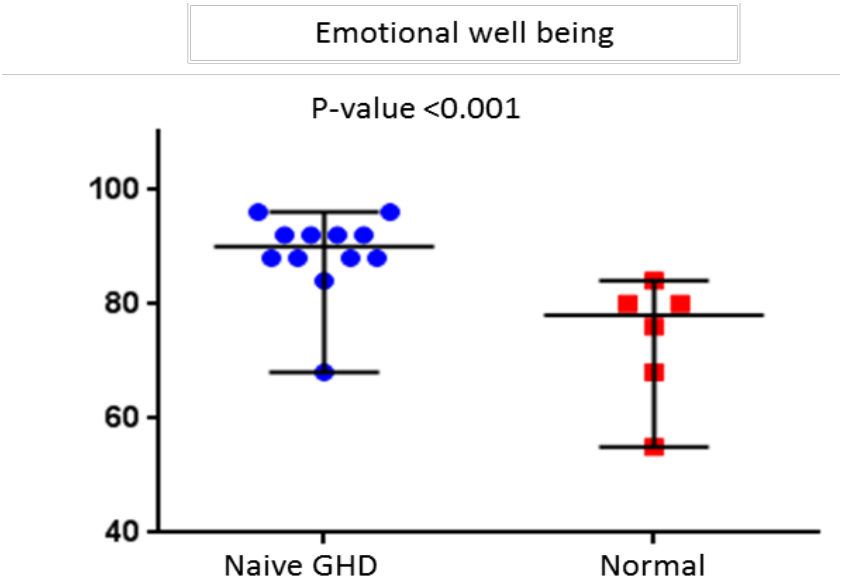

Figure 7-6 Individual data of emotional wellbeing aspect of SF-36 QoL in the first time assessment
(Higher scores reflect better QoL)

7.5.3 AGHDA QoL scores in the retesting group

Among the retesting group, there were no statistically significant differences in total QoL-AGHDA scores between those who were have persistent GHD and those who were GH sufficient after retesting (6 points (2, 8) persistent GHD vs. 9 points (7, 17) GH sufficient, respectively, p=0.10), Table 7-5, Figure 7-7. The worst QoL-AGHDA score (17) was recorded in patient ID 7 (IGHD-Obesity (BMI SDS=3.1) who was considered GH sufficient after retesting. However, unexpectedly, all GH sufficient patients tended to have a poor QoL across most of aspects of AGHDA and they significantly lacked energy and complained of tiredness compared to those with persistent GHD (1 point (0, 1) persistent GHD vs. 5 points (3, 5) GH sufficient, p= 0.03) as seen in Figure 7-8.

Table 7-5 AGHDA scores of the retesting group.
(Higher scores reflect poorer QoL)

Subscale	Persistent GHD (n=5)	GH sufficient (n=3)	P-value
Total scores(25)	6 (2, 8)	9(7, 17)	0.10
Memory and concentration(6)	1(0, 3)	3(0, 4)	0.55
Tiredness (7)	1(0, 1)	5(3, 6)	**0.03**
Tenseness (3)	1(0, 2)	2(0, 3)	0.55
Social isolation (5)	2(0, 3)	1(0, 2)	0.45
Self-confidence (4)	1(0, 1)	1(0, 3)	0.65

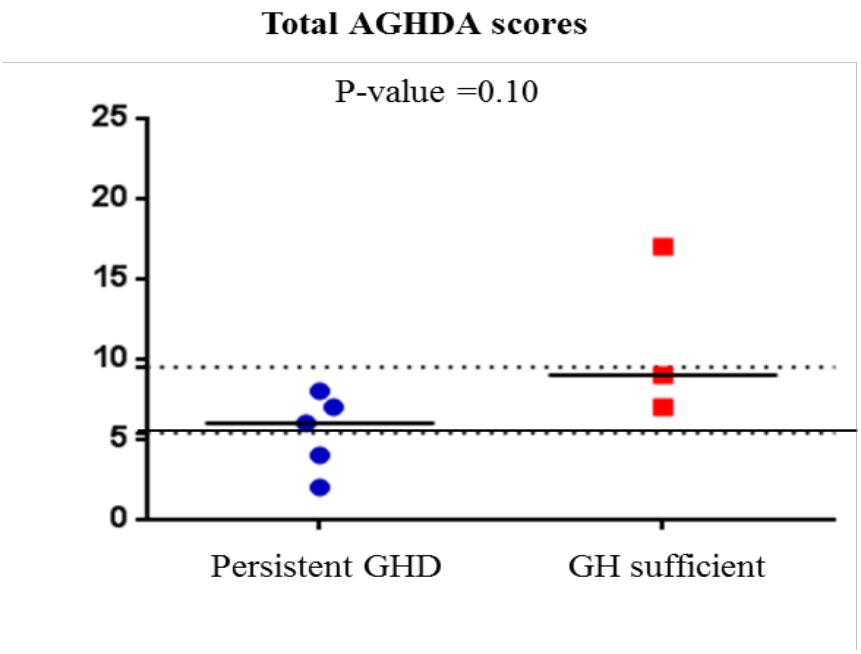

Figure 7-7 Individual data of total AGHDA QoL in the retesting group.
(Higher scores reflect poorer QoL)

——— 5.4 reference line for the UK general population (aged 18-25 years) (490)

- - - - - - 9.5 reference line for young adult with CO-GHD in UK (264)

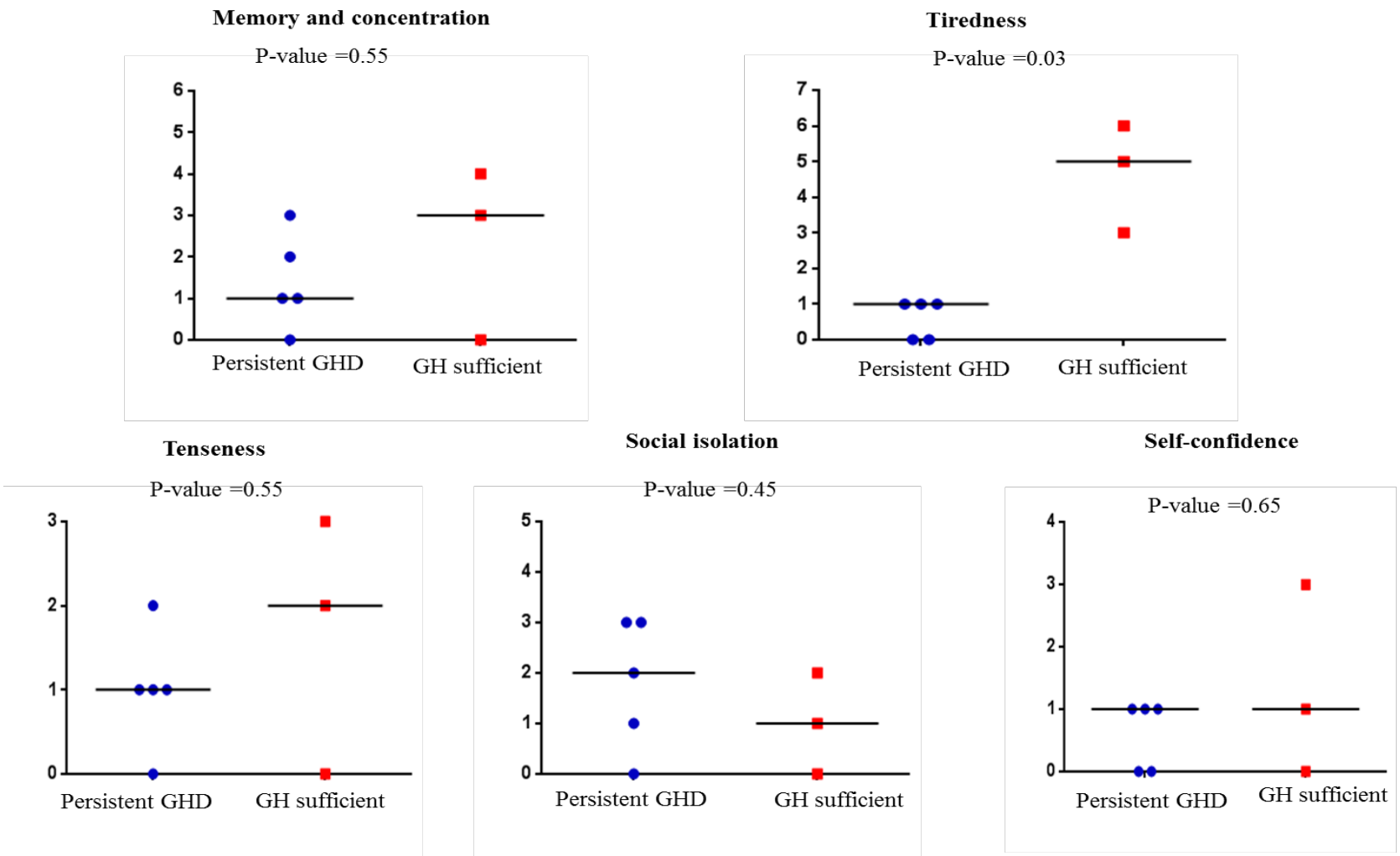

Figure 7-8 Individual data for five subscales of AGHDA QoL in the retesting group.
(Higher scores reflect poorer QoL)

7.5.4 Correlation between QoL and clinical data

There were no correlations between anthropometric and clinical parameters (age, sex, weight, height, and BMI) and SF-36 total scale or subscale of first time assessment group. However, GH peak levels were correlated positively with the scale of level of energy and fatigue ($r=0.59$, $P = 0.04$), but negatively with the scale of emotional well-being ($r=-0.68$, $P<0.001$), Figures 7-9, 7-10. No correlations were found between total and subscale scores of SF-36 with either DXA or tibia bone and body compositions parameters (data was obtained from chapter 5). In addition, there were no correlations between total and subscales scores of SF-36 and muscle function parameter measured by mechanography (data was obtained from chapter 5, only seven subjects of first time assessment had their muscle function assessed).

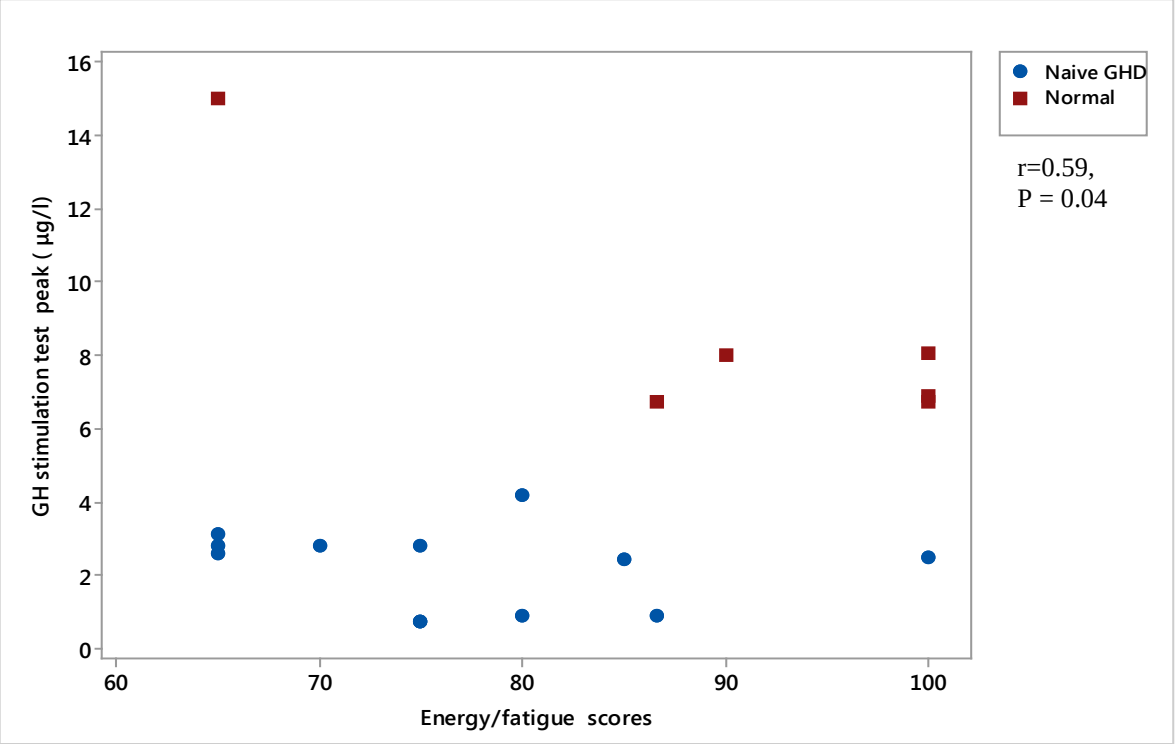

Figure 7-9 Spearman`s correlation between GH peak and aspect of energy/fatigue in SF-36 QoL of the first time assessment group

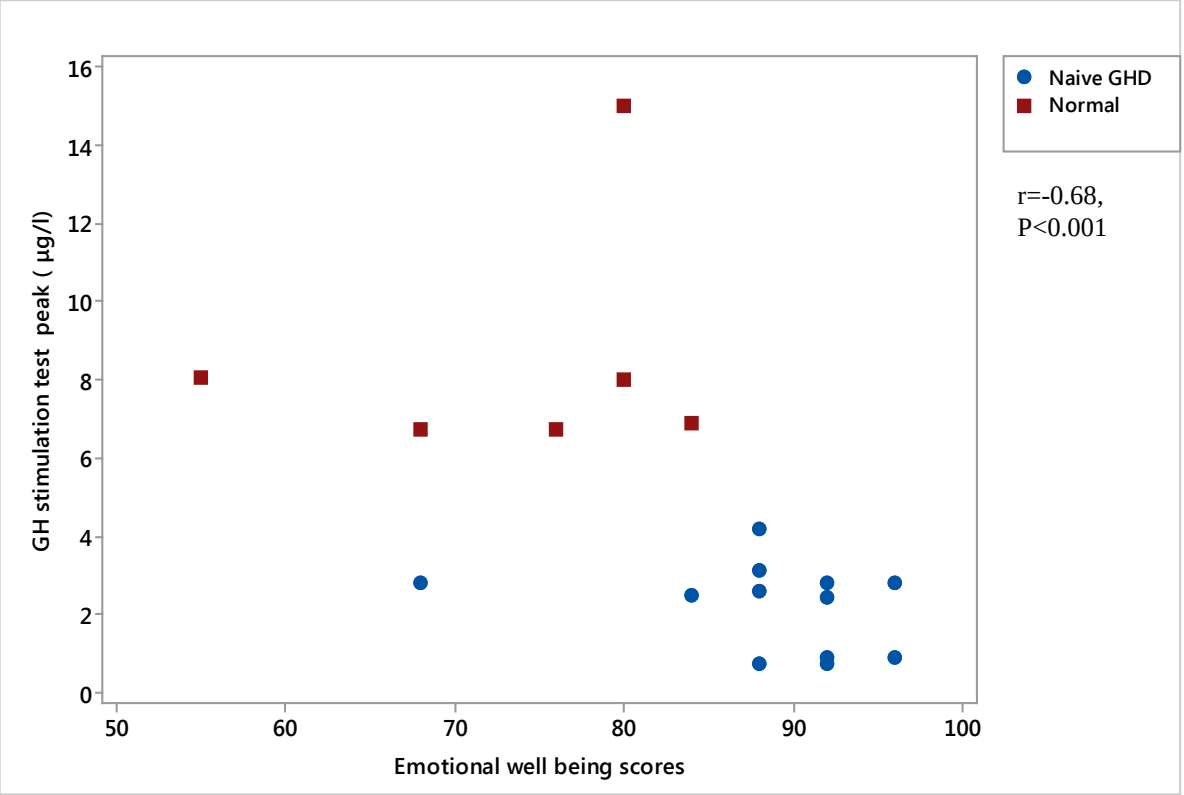

Figure 7-10 Spearman`s correlation between GH peak and aspect of emotional wellbeing inSF-36 QoL of the first time assessment group

With regard to the retesting group, there were no correlations between anthropometric and clinical parameters at time of retesting (age, sex, weight, height, BMI, duration of childhood rhGH and duration of discontinuation of rhGH) and total scale or subscales of AGHDA. Furthermore, there were no correlations between total AGHDA with either GH peak or IGF-1 levels on retesting. However, we found a significant negative correlation between IGF-1 levels at time of retesting and memory/ concentration scores (r=-0.65, p=0.04) indicating that a lower level of IGF-1 corresponds to poorer scores of memory and concentration, Figure 7-11.

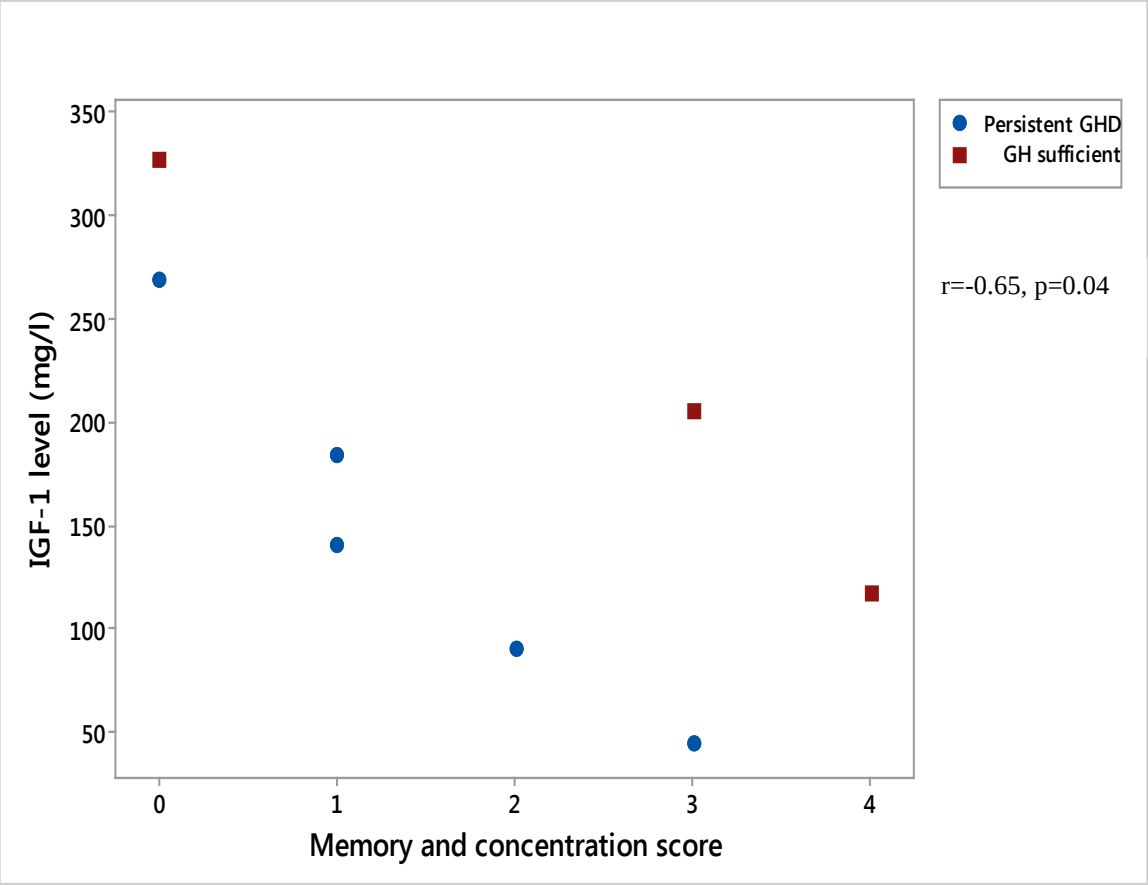

Figure 7-11 Spearman`s correlation between retesting IGF-1 levels and aspect of memory and concentration in AGHDA in the retesting group.
(Higher scores reflect poorer QoL)

7.6 Discussion

In this chapter we compared QoL in subjects with CO-GHD subjects with similar height but with normal GH levels subjects. Our data showed that QoL was not substantially altered in patients with CO-GHD at time of initial evaluation or retesting at final height. In our study, however, naive GHD reported lack of energy and tiredness at time of diagnosis (491). However it is unknown whether this impairment is implicated directly to lack of energy due to GHD or that partly caused by muscle weakness of GHD. Conversely, emotional wellbeing was poorer in normal subjects compared to naive GHD. This finding is similar to previously reported in short stature children with and without GHD (476,482) suggesting that short stature and not GHD per se is associated with reduced QoL in childhood. However, other studies reported that there is no evidence of impaired QoL in short stature children and adolescents with or without GHD (492), suggesting that other factors other than height and GH levels may have impact on QoL (493). A large population-based study reported short stature children did not differ from their non-short peers in a range of social, emotional and behavioural outcomes (494), when short stature in adult life may be associated with a significant reduction in health related QoL (495). In the same context, an increase in self-esteem has also been reported in a previous study of rhGH treatment in GHD children (491,496), but not in children with idiopathic short stature who were rhGH-treated (497). It is of note; however, the proxy reports of SF-36 in our cohort would be considered the parents' own functioning and well-being in this finding. Research on the relationship between children's self-reports and parent proxy reports show parents of short children commonly report problems of cognitive development, personality, self-esteem or social relations (492), and parental perceptions to seek health care for their child are most likely to influence their child functioning and well-being (498).

The QoL-AGHDA disease specific instrument was developed particularly to measure QoL in adults with GHD with limited studies in adolescents with CO-GHD at final height (232,274). The mean QoL AGHDA scores for the UK general population (aged 18-25 years) is 5.4 (95% CI: 4.91–5.98) (490), and for patients with CO-GHD is 9.5 (95% CI: 8.81–10.20) (264). Our retesting group showed overall AGHDA scores within the range of the mean QoL AGHDA scores for the UK general population with no significant differences between the medians scores of those who have persistent GHD and those who were GH sufficient after retesting. The finding of the present study is not unprecedented. Several previous large longitudinal studies evaluated the effects of discontinuation and resumption of rhGH treatment on QoL elements in young adults with CO-GHD demonstrating no changes in QoL in adolescents with CO-GHD as measured at baseline when they stopped rhGH after final height and then re-measured after two years either being off GH or re-commencing rhGH therapy (229,232,247). Conversely, other studies demonstrated that discontinuation of rhGH treatment leads to a decrease in QoL within 6 months, which is counteracted within 3-6 months after restarting rhGH therapy (276,277).

Surprisingly, our subscale analyses revealed paradoxical and unexpected results with those who were GH sufficient after retesting feeling significantly more tired and having poorer energy and

vitality scores than those who had persistent GHD. This finding suggests there are possible other unknown confounders which interfere with energy levels and tiredness rather than GH status in our cohort. From the literature, untreated adults with CO-GHD reported to have low energy levels, vitality, mental fatigue, emotional reactions and social isolation (499). In particular, the increased fatigue was a key element associated with GHD which substantially diminishes quality of life (499). Therefore, it can be assumed that in previously CO-GHD particularly those who were considered partially GHD (GH peak > 5 μg/l but < 10 μg/l) QoL may be still affected, however, to a lesser extent than those who were conformed persistent GHD (500). This finding indicates the importance of follow up and reassessment in those who were CO-GHD and are then no longer GH deficient after retesting.

In the present data of the retesting group, GH peak levels at retesting were not correlated with AGHDA scores. However, lower IGF-1 levels were linked with poorer memory and concentration performance. This finding assumes that the IGF-1 levels seem a more valuable predictor of the impact of rhGH discontinuation on QoL as was reported previously (276,277,501), though the underlying mechanism is not fully understood. Further prospective studies to elucidate this question are needed to measure QoL while still on rhGH and after a period off rhGH.

Nevertheless, there are a number of issues that should be considered to justify our findings in the retesting group. Firstly, a small sample size that we may not have enough data to compute the effect of discontinuation of rhGH after final height in adolescents with CO-GHD. Another issue to consider, is the short duration of time off rhGH in our cohort which it means it was probably too early for the perception of a decline in QoL to emerge as previously stated (242). In addition, AGHDA-QoL is a self-rating questionnaire that may underestimate the true impairment of QoL (502), and it could lack the sensitivity in recognizing impaired QoL in this particular population of adolescents and young adults with CO-GHD (500,503).

Generally, this study has several limitations that should be acknowledged since they might negatively affect the interpretation of the results. In addition to the small sample size, this is only a cross-sectional baseline analysis. It would be ideal to analyse QoL longitudinally in GHD children before, during and after initiation of rhGH treatment to see whether the reason for these effects lies in GH itself or in other factors remains to be determined in further investigation. Another limitation to consider is that the QoL measures tools (SF-36- AGHDA) may lack sensitivity to detect the differences between our studied groups, as the existing measures have not been developed for, or validated within the population of children and adolescents with CO-GHD.

7.7 Conclusion

In summary, our study demonstrated normal ranges of overall QoL aspects in children and adolescents with CO-GHD compared to subjects of same stature but with normal GH levels. Naive GHD subjects scored lower in energy and vitality but higher in the subscale emotional well-being than normal subjects, whereas GH sufficient adolescents with CO-GHD have reported significantly poorer scores on the scale of energy and tiredness than those with persistent GHD after retesting. Further large longitudinal studies using more specific validate instruments are needed to assess QoL in children and adolescents with GHD before and after initiating rhGH treatment.

CHAPTER 8

The Effect of Weight Bearing Exercise in Children and Adolescents with Childhood-Onset Growth Hormone Deficiency

8.1 Abstract

Background: Childhood onset growth hormone deficiency (CO-GHD) is a disorder that impacts several aspects of individual health throughout life. Growth hormone replacement therapy (rhGH) during childhood has been used to increase growth and final height in children with CO-GHD. However, rhGH has other benefits on bone health and metabolism and may be useful for a longer period during the transition from childhood to adulthood. There is also some evidence that weight bearing exercise (WBE) can optimize bone health and development during childhood and adolescence.

Aim: To assess the effect of weight bearing exercise on the bone health of children and adolescents with CO-GHD with or without GH replacement therapy.

Patients and methods: This was a prospective pilot study of 14 subjects among a first time assessment group (age 5 to 13.8 years) and five subjects with CO-GHD among a retesting group (age 15.2 to16.9 years). They were randomised to an exercise program (EX) (25 jumps off 25 cm platform step/ three days /week for six months) or no exercise (control), in addition to rhGH as prescribed. Measurements were performed at baseline and six-months assessing bone health and body composition by imaging (DXA and p.QCT) and biochemical assessment.

Results: Of the 14 subjects among the first time assessment group (10-GHD, 4- normal), eight subjects were allocated EX (compliance rate median (range) is 33% (7-80)) alone or combined with rhGH. Over the study period, TB/LS- BMD, BMC and BA have increased in all subjects. However, TB/LS BMC for bone area SDS tended to decrease in all subjects, but remained within +2 SDS. There was relative positive gain in LM in both GHD with rhGH alone and rhGH combined with EX, percentage change (PC %) range ((13.3%, 23.5%), (10.3%, 28.6%) respectively). Of the remaining group, the two GHD subjects on EX alone and the normal subjects had positive PC% in LM ranged from 2.5% to 19.4%. FM decreased in three of the five subjects who were on rhGH alone (ranged: -24% to -16%) and in all three who had combined rhGH with EX (ranged: -44% to -15%). However, FM noticeably increased in those who had GHD with EX alone (111%, 80%), with no obvious change in normal subjects. In the retesting group, of the five retesting group subjects (4-persistent GHD (3 received rhGH), one GH sufficient) three were allocated to have EX (compliance rate is 26% (16-83)) either alone or combined with rhGH as required. All subjects had steady bone density with no noticeable changes from baseline to follow up, and all had a value of TB/LS BMC for bone area SDS between +2 SDS. LM tended to increase in all patients, FM did not change obviously in most patients, with only one subject (GH sufficient on EX alone and high compliance rate (83%) losing 11.4% FM from baseline.

Conclusion: There was insufficient data to recommend the use of WBE in the absence of rhGH in children and adults with CO-GHD. However, WBE may be more beneficial combined with rhGH in these subjects and that require further large studies to explore the interactions between rhGH replacement and exercise on bone health of CO-GHD.

8.2 Introduction

Patients with growth hormone deficiency (GHD) exhibit clinical and biochemical abnormalities involving alteration in the muscular skeletal, the cardiovascular system, lipid metabolism and quality of life (133,247). Until quite recently, the management of children with childhood onset-GHD (CO-GHD) had focused on the use of recombinant human growth hormone (rhGH) therapy to maximise adult height, which most adolescents achieve in the middle of the second decade of life. However, it is becoming increasingly recognised that rhGH has other benefits during childhood and may be useful for a longer period during the transition from childhood to adulthood for optimising other aspects of health (247,358). Based on these suggestions, a handful of studies, supported by the pharmaceutical industry, have been performed to explore the benefits of rhGH on bone health and body composition after attaining final height and transition. In some placebo controlled studies, rhGH therapy has been reported to have had a favourable effect on BMC, BMD, LM, and FM (227,229) whilst another has not shown any significant effect (232).

On the other hand, it is well documented that mechanical loading is a major regulator of bone mass and geometry and contributes to a large part of BMC accretion in weight-bearing bones (504). A handful of studies have been carried out on children and adolescents and confirmed that weight-bearing exercise (WBE) (in particular jumping exercise) appears to enhance bone mineral accrual and improve bone strength by maximising peak bone mass (281,286,287). Weight-bearing activities have been also found to contribute to favourable benefits to body composition and regulation of body metabolism (342). Additionally, it has been suggested that physical activity itself may be directly associated with measures of health related quality of life and the feeling of well-being (356).

In view of the above, more interesting evidence from animal models showed that the combination of rhGH and mild exercise has markedly enhanced bone density (505). However, it is unclear as to whether WBE with/without rhGH has any effect on bone mass, body composition and quality of life in subjects with CO-GHD. The role of exercise in altering these health outcomes and altering the need for therapy with rhGH deserves further exploration.

8.3 Aims and Hypotheses

Through the present pilot study, we aim to collect preliminary data that examines the short-term effects of a supervised WBE regimen with /or without rhGH on bone health, in addition to body composition, metabolism and quality of life.

8.3.1 Hypotheses

1. Bone health is adversely affected in patients with CO-GHD.
2. Exercise mitigates the effect on bone health in CO-GHD patients
3. The beneficial effect on bone health is greater in those who have exercise and rhGH.

8.3.2 Primary outcome measures

Change in dual x-ray absorptiometry (DXA) based total body (TB) and lumber spine (LS) bone mineral content (BMC).

8.3.3 Secondary Outcome Measures

To investigate the effects of rhGH and exercise intervention on

a. Change in markers of bone turnover

b. Markers of glucose homeostasis

c. Change in body composition

d. Change in quantitative computed tomography (pQCT) based Tibial vBMD and Cortical Thickness

e. Quality of life measures (AGHDA, SF-36)

8.4 Study Methodologies

8.4.1 Study design and subjects

Open intervention, randomised controlled, study design and data collection have been illustrated in Figure 8-1. Inclusion and exclusion criteria were described in chapter 5.

8.4.2 Sample size and power

This is a pilot study aimed to study the effects of a jumping exercises program on TB and LS - BMC in patients with CO-GHD (primary end point). Literature indicated favourable significant change in LS-BMC [ranging from 0.9% to 3.9%] of jumping exercise interventions in healthy children and adolescents in a seven month average period compared with controls (284,286,327). Since no prior research existed regarding the impact of jumping exercise training among participants with CO-GHD, it is therefore difficult to calculate the sample size adequately.
For this preliminary pilot study, there will be four groups of patients - those who are randomised to exercise will consist of some with GHD (exercise-GHD) and some who will have normal GH secretion (exercise- normal). Those who will act as a control group will also consist of children with GHD (control-GHD) and those with normal GH secretion (control- normal). We assumed that there will be approximately 50 eligible subjects who newly receive rhGH treatment each year in the Royal Hospital for Children as well as adolescents who are retested for GHD after attaining final height. It is anticipated that 60 patients will be recruited over 1.4 years. With a total sample size of 60 participants, a convenience sample of around 15 subjects in each group was deemed to be sufficient to characterise differences in LS/TB BMC.

8.4.3 Ethics

The study was approved by the National Research Ethics Service and informed consent was obtained from all parents and children, where appropriate.

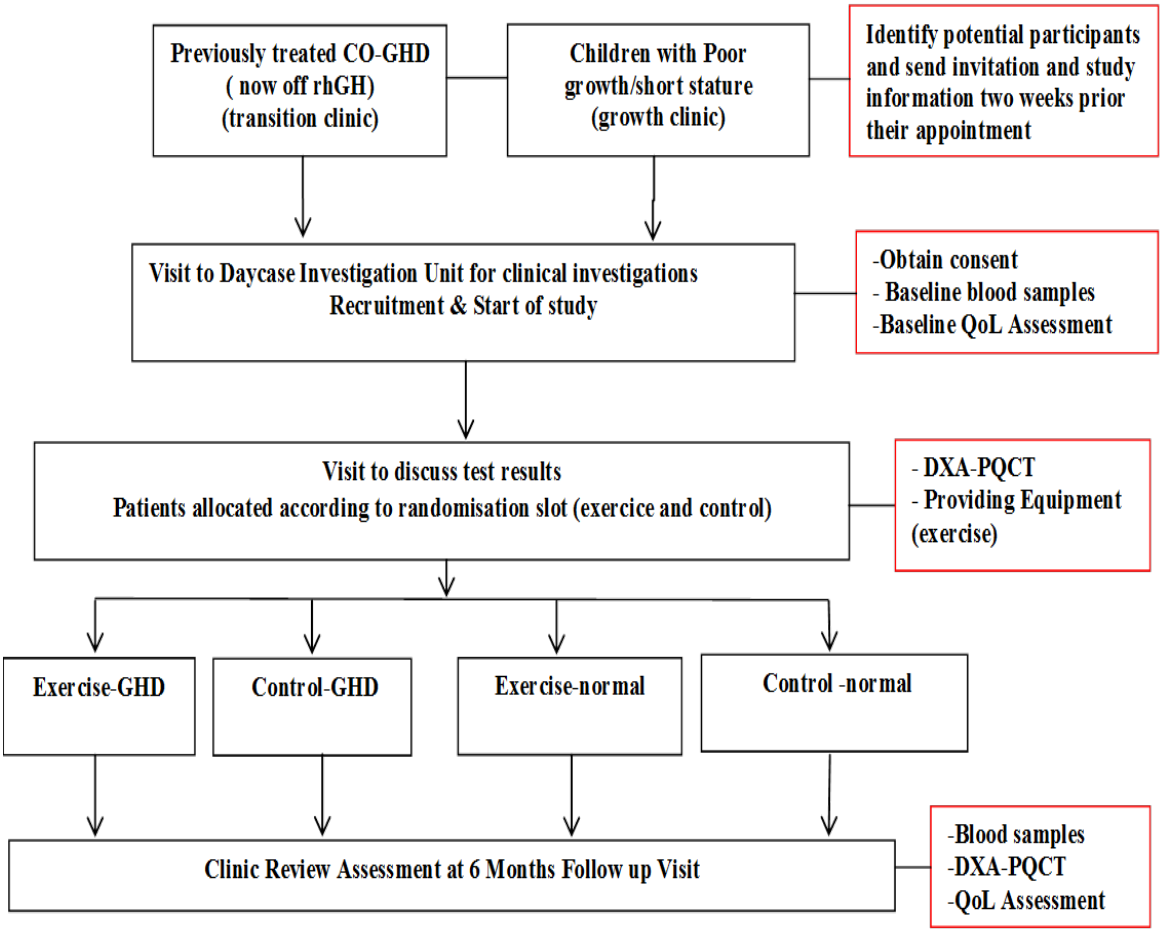

Figure 8-1 Study design and data collection protocol

8.4.4 Participants

Potential participants (who were being referred either for assessment of poor growth /short stature in childhood or for retesting of CO-GHD in adolescence after completing final height and off rhGH) were identified from the endocrine nurse appointment list diary. A study invitation letter with appropriate information sheets was posted two weeks prior to their appointments. On the day of testing, potential participants were provided further details and explanation of the study and ascertained whether they were interested in taking part in the study. When the participants (and their care giver) agreed to participate in the study and met inclusion criteria, written informed consent was obtained from each participant (and their care giver).

8.4.5 Randomisation

Randomization was performed prior to recruitment using the random function in Excel with a block of 10 and 5 in each arm to ensure approximately equal size of study groups and that was repeated once the first set was allocated.
After all inclusion and exclusion criteria were checked, and informed consent given, patients were assigned to either intervention (exercise) or control (no exercise) arms according to the schedule of randomization results in Excel.

8.4.6 Exercise Regimen

The home-based exercise program consists of jumping off a 25 cm high platform step (Reebok step with safe anti-slip surface, Figure 8-2) up to 25 jumps/a day three times a week for 6 months. We assumed jumping off a 25 cm step height could generate ground reaction forces of 3 to 4 times body weight (506). To standardise the exercise, each participant was instructed to jump as high as they can off the box and land with his/her knees slightly bent (instruction leaflet was provided, appendix D). The exercise program was recorded using provided video camera by children or their caregiver as appropriate.
All participants were asked to start the exercise immediately or with starting rhGH when it was prescribed.
The control group would receive their usual medical care and advice and undergo the same investigations as the intervention group

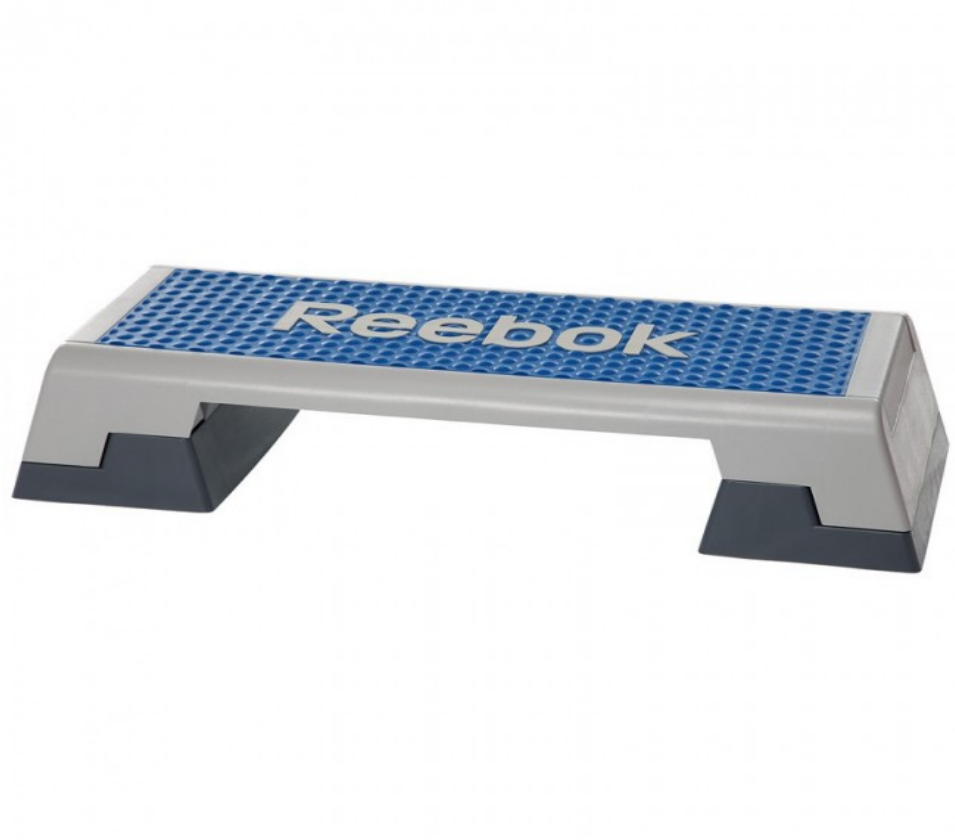

Figure 8-2 Reebok Step.

Bubble surface for no-slip feet on the workout surface, adjustable to three levels (15 cm, 20 cm and 25 cm).

8.4.7 Compliance

Compliance percentage was calculated from the video diary (using provided Vivitar DVR638 HD Camcorder) as number of sessions completed divided by the 72 possible sessions (3 times/week/6 months) x 100.

8.4.8 Biochemical assays

Fasting blood samples were taken to coincide with routine clinic visits after overnight fasting at baseline and follow up visits. Bone profiles and elements (Calcium, phosphate, magnesium, parathyroid hormone (PTH) - vitamin D (25 (OH) Vit-D)) and lipids profiles were measured immediately after blood sampling in a Biochemistry laboratory in RHSC with standard methods. The remaining samples were centrifuged at 2600-2800 rev/minute for 10min, and the serum was subsequently stored at -70C until the assays were performed (bone biomarkers-adipokines-F-insulin-FFA) as described in chapters 5 and 6.

8.4.9 Bone densitometry and geometry

DXA scan (Lunar Prodigy, GE Medical Systems, and Waukesha, Wisconsin, USA) was performed to assess bone parameters and body composition. To minimise the size effects in DXA bone densitometry, bone and body composition parameters were corrected for bone area/height/age as described in chapter-4. Similar to chapter 5, measurement of TB/LS BMD Z scores $_{\text{height age}}$ and BMC for bone area SDS were excluded in children aged $\leq$6 years.
Tibial volumetric BMD, geometric and surrogate markers of bone strength were also determined by a peripheral quantitative CT scan (pQCT) (Stratec XCT 2000, Software version 6.00, Pforzheim, Germany) at the 4% , 38% and 66% site and stress-strain index (SSI) (mm3) at (38%). In addition, lean and fat areas were assessed at the 66% site. The pQCT bone outcomes were converted to Z-scores relative to age, sex and height based on recent references data (412). The pQCT scans with any movement artifacts and other potential problems were excluded.

8.4.10 Quality of life measures

As described in chapter 7:
1- Short form-36 (SF-36) questionnaire was used for first time assessment at baseline and follow up. The scores were transformed to values between 0 and 100, with higher values indicating better QoL.
2- A questionnaire Quality of Life Assessment of Growth Hormone Deficiency in Adults (AGHDA) was used for retesting the group at baseline and on follow up. The ‘yes’ responses constitute a score, with a higher score denoting a poor QoL.

8.5 Statistical Analysis

The outcome variables were expressed either as percentage change between the baseline or follow up ((follow up-baseline)/baseline) *100) or absolute change (follow up-baseline). Delta change in height SDS was calculated as [(height SDS at follow up - height SDS at baseline) / (age at follow up - age at baseline)]. Spearman correlations were used as appropriate to determine associations between variables. All statistical analyses were performed using the Minitab17 software (Minitab, Coventry, UK), with significance set at a level of 5% ($P<0.05$). All graphs were performed using prism 5- GraphPad.

8.6 Results

8.6.1 Study population

Of the 63 children approached during the study period (Aug-2012-Nov-2013), 36 were recruited and agreed to take part in the study, and 27 declined for a range of reasons (Figure 8-3).
The 36 patients (n=25 were in the first time assessment group of children, and n=11 were in the retesting group of adolescents with CO-GHD), were allocated according to randomisation into either carrying out WBE or acting as controls.
Of the n=18 assigned to the exercise group (n=9 GHD: n=9 normal), only n=9 had completed the study. Of the remaining n=9 who were dropouts, four children withdrew from the study even before starting the exercise regimen (one with persistent GHD was lost at follow up and when she returned, it was out of the study timeframe)- (three children were never returned after their normal GH results), four children were lost at follow up after starting the exercise regimen (three subjects with normal-GH levels who failed to attend follow up appointments, and one naive GHD was lost to follow up as well); another child was excluded as she suffered from deterioration of a non-related chronic illness.
In the n=18 control group (n=12 GHD: n=6 normal), n= 10 patients had completed the study. The remaining n=8 were dropouts, five children with naive GHD declined to participate in the study because they moved to other centres(3) or they were not willing to have rhGH treatment(2), and three with normal were also discharged from the clinic and contact was lost.
The overall study drop-out rate was high (47%) and was caused by lost to follow-up of the study cohort particularly those with normal GH results who had a high “Did not Attend” (DNA) clinic rate. From figure 3, amongst the subjects who completed the study, n=14 (n=6 exercise - n=8 controls) were in the first time assessment group. Of the remaining, n= 5 (n=3 exercise: n=2 control) were in the retesting group of adolescents with CO-GHD.

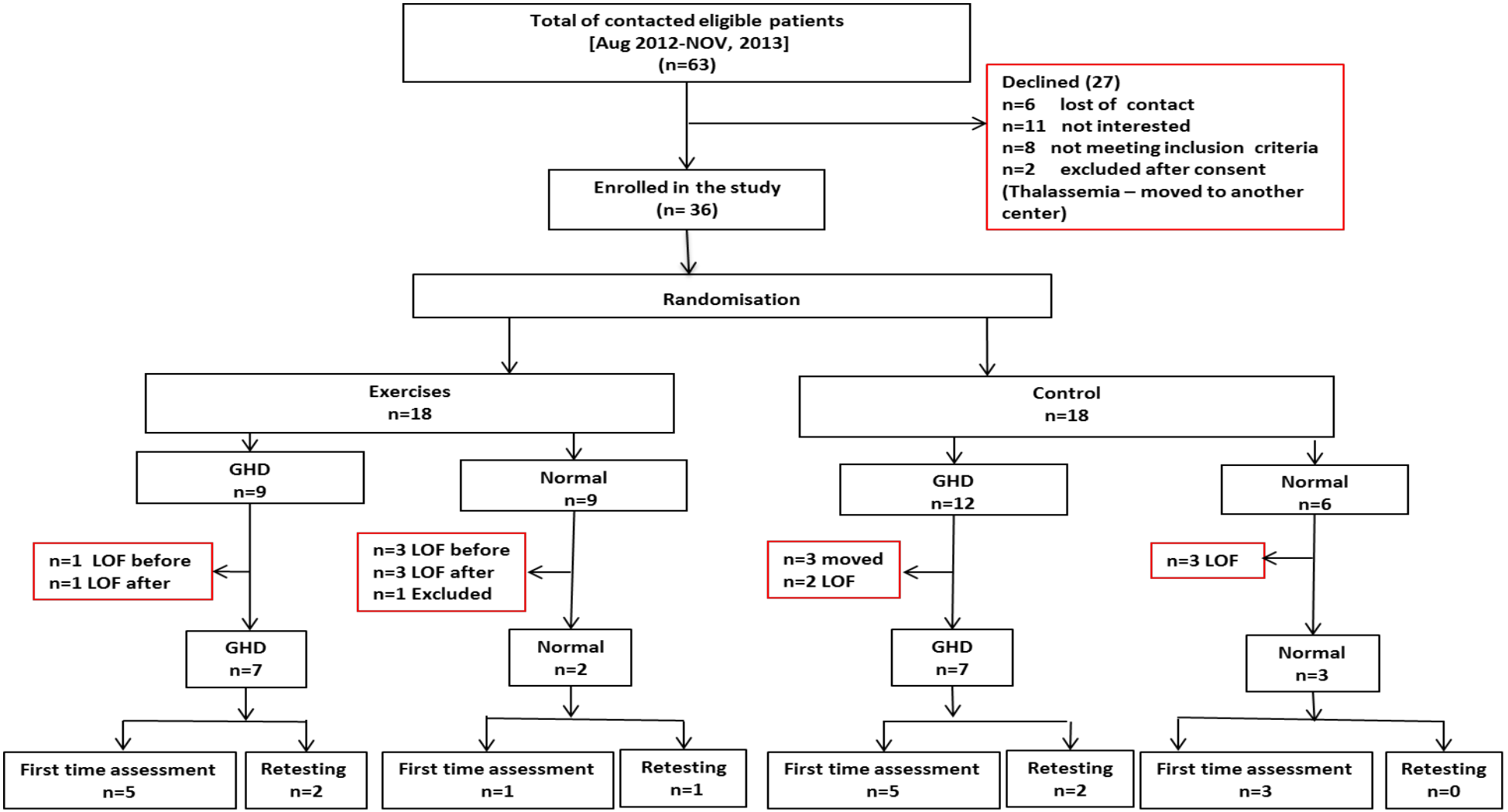

Figure 8-3 Flow diagram of study recruitment.

N: number of subjects; LOF before: loss of follow up before starting exercises; LOF after: loss of follow up after starting exercises; GHD: growth hormone deficiency

8.6.2 Compliance

Of the nine patients randomized to the exercise regimen and who completed the study, two patients had returned their video diary; their compliance was calculated depending on recorded sessions. The other two had not returned the video diary but reported that they had only missed six sessions or fewer. Three patients had not recorded any sessions and self-reported (or by their parents) that they had performed three sessions per week only in the first month then stopped (lost interest and motivation – became busy and got new job, one parent was sick and not able to continue). Of the remaining two, one had performed only one session every week over the study period (weekend day only) and the other had less than one session every four weeks over the study period. The median of overall compliance in the first time assessment group is 33% (7- 80) and in the retesting group is 26% (16- 83).

No adverse events attributable to the jumping exercise program were reported.

Due to the high dropout rate, small sample size, poor compliance and heterogeneity of subjects in each arm, we could not test the exercise intervention per se; hence we attempted to isolate the effects of rhGH treatment or exercise, and both, by individual data in each group (first time assessment group- retesting group), thereby limiting the effects of variability in receiving rhGH with exercise compliance.

8.6.3 First time assessment group

8.6.3.1 General characteristics

Fourteen subjects who completed the study belonged to the first time assessment group (age range 5.6, 15.6 years: 11 boys); ten of them were confirmed with naive GHD and four with normal GH levels (Figure 8-4).

Of those, n=10/14 (eight with GHD, two with normal GH levels) have started rhGH (paediatric dose: 25-35ug/kg/d). According to randomisation of the exercise program (EX) and starting rhGH, of these 14 participants, five with GHD had only rhGH, three with GHD had combined rhGH and the exercise, two with GHD had only the exercise, one normal with normal had combined rhGH with the exercise, one normal had only rhGH, and two normal had neither rhGH nor exercise. The median duration from baseline to follow up for the whole cohort is 0.9 yr (0.6, 1.5).

Individual data of the first time assessment group who completed the study are shown in Table 8-1.

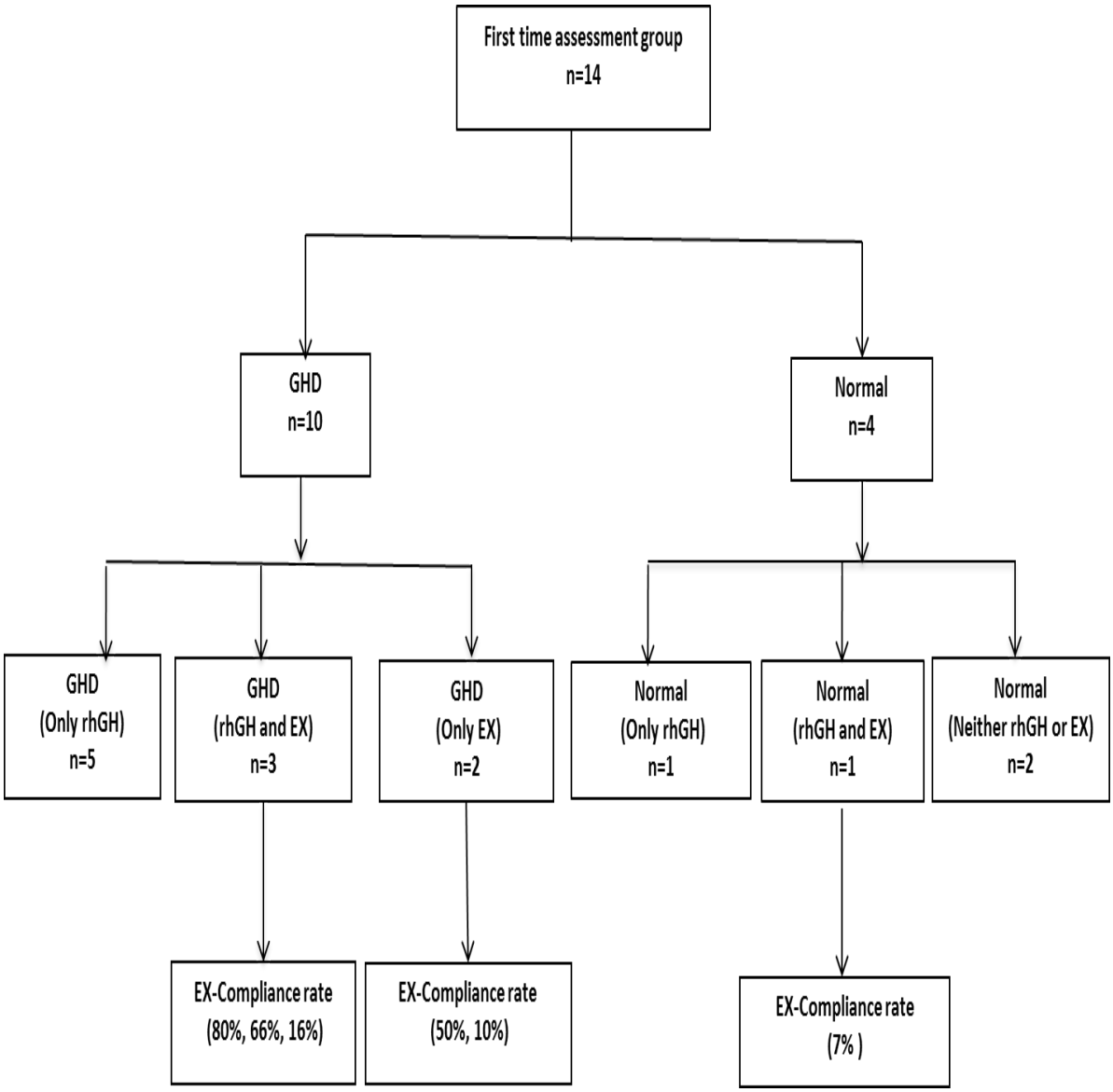

Figure 8-4 Flow chart of the first time assessment.
rhGH: recombinant human growth hormone; EX: exercise

Table 8-1First time assessment group individual demographic and clinical characteristics.
*F stands for first time assessment; ID: patient identification; rhGH: recombinant human growth hormone; EX: exercise

	Group	ID	Age at BL (yrs)	GH-peak (µg/l)	Pituitary MRI	rhGH (yes, no)	Exercise compliance (%)	follow up (yr)	Intervention (yr)	Habitual P.A hrs/W	Co- morbidities /medications
GHD	Only rhGH	F1	9.2	3.5	-	Yes	Control	0.8	0.6	-	Trisomy 21
		F2	8.2	3.1	Normal	Yes	Control	1.0	0.9	-	-
		F3	10.0	0.9	Ectopic posterior pituitary	Yes	Control	0.9	0.9	-	-
		F4	12.3	2.5	Normal	Yes	Control	0.9	0.8	3 (football)	Tourette's syndrome (ADHD)
		F5	10.9	2.8	Arachnoid cyst	Yes	Control	0.6	0.5	6 (football)	-
	rhGH with EX	F6	5.6	2.4	Ectopic	Yes	EX (80%)	0.8	0.6	1.5(swimming)	-
		F7	14.9	0.7	Empty sella	Yes	EX (66%)	0.7	0.7	-	-
		F8	10.2	2.8	craniopharyngioma	Yes	EX (16%)	1.3	0.9	-	-
	Only EX	F9	11	2.8	Neuro-glioma	No	EX (50%)	0.9	0.8	-	Neurofibromatosis – Precocious puberty
		F10	10.9	4.2	Idiopathic	No	EX (16%)	1.5	0.5	-	-
Normal	rhGH with EX	F11	5.8	8.0	-	Yes	EX (7%)	0.6	0.5	-	45/46 XY gonadal dysgenesis
	Only rhGH	F12	15.6	22.3	-	Yes	Control	0.8	0.5	-	Juvenile rheumatoid arthritis (Predenisolone-methotrexate-sulfasalazine-abatacept)
	Neither rhGH or EX	F13	5.8	6.8	-	No	Control	0.9	-	2 (Dance class)	-
		F14	14.4	18.8	-	No	Control	0.8	-	2 (football)	Mitochondrial disease with primary ovarian failure

8.6.3.2 The anthropometric characteristics from baseline to follow up

The individual demographic characteristics of the first time assessment group who completed baseline and follow-up assessments are shown in Table 8-2.

Positive delta (Δ) changes in height SDS were observed in all subjects with GHD who received rhGH ranging from (0.4 to 1.0) in rhGH alone, (0.4 to 0.9) in rhGH with EX, but a small change in height SDS Δ was recorded in those who were GHD on only EX (-0.1 and 0.2). In normal subjects of the first time assessment group, patient's ID (F11) who received rhGH with EX showed the highest positive Δ SDS (Δ=2.2) compared with rhGH alone (patient's ID (F12) Δ= 0.3), or either (patient's IDs; F13 Δ = -0.1, F14 Δ= 0.0), Figure 8-5.

Weight and BMI were not obviously changed over the study period in the present cohort apart from three subjects [(Patient's ID; F3 (GHD on rhGH alone) - Patient's IDs F9 and F10 (GHD on EX alone)] who showed high percentage change (PC %) in their weight and BMI.

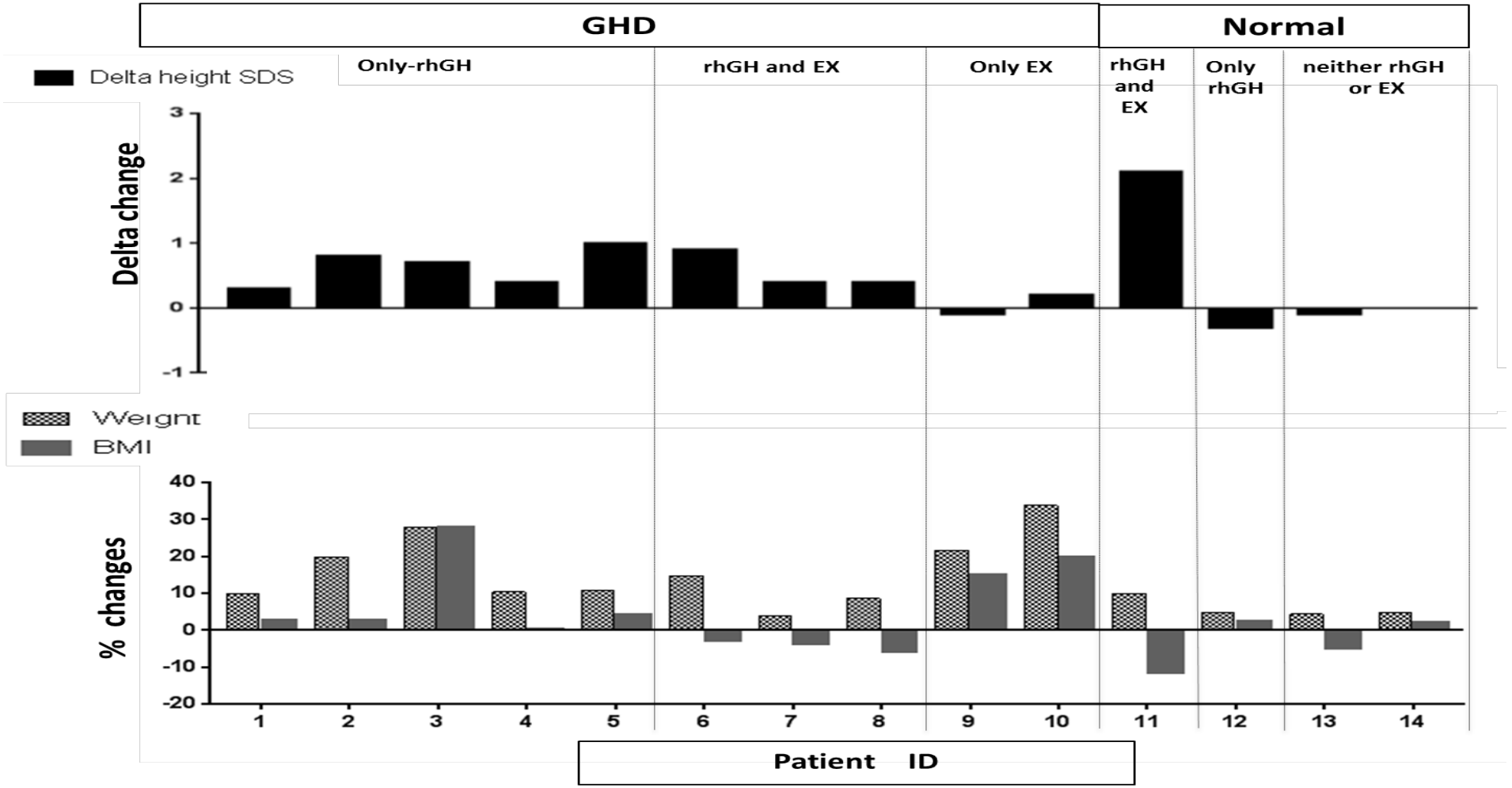

Figure 8-5 Individual data of delta height SDS and percentage changes in weight and BMI of the first time assessment group.

Table 8-2 Demographic characteristics of the first time assessment group

	Group	ID	Time	Height (Cm)	Height-SDS*	Weight (Kg)	Weight SDS	BMI	BMI SDS	Tanner stage
GHD	Only rhGH	F1	BL FL PC%	106.4 110.0 3.4	-3.4 -3.2 0.3	18.6 20.4 9.7	-3.2 -3.2 -	16.4 16.9 2.4	0.0 0.0 -	I I -
		F2	BL FL PC%	110.0 118.9 8.1	-3.43 -2.6 0.8	17.2 20.6 19.8	-3.5 -2.6 -	14.2 14.6 2.5	-1.2 -1.1 -	I I -
		F3	BL FL PC%	128.6 137.7 7.1	-1.5 -0.9 0.7	32.4 41.4 27.8	0.2 1.0 -	19.6 25.0 27.8	1.4 1.8 -	I I -
		F4	BL FL PC%	133.5 140.0 4.9	-2.2 -2.0 0.4	26.3 29.0 10.3	-2.67 -2.5 -	14.8 14.8 0.3	-1.8 -2.1 -	I I -
		F5	BL FL PC%	129.3 133.4 3.2	-2.0 -1.7 1.0	26.6 29.5 10.9	-1.7 -1.2 -	15.9 16.6 4.2	-0.5 -0.3 -	I I -
	rhGH with EX	F6	BL FL PC%	97.7 106.1 8.6	-3.3 -2.6 0.9	15.0 17.2 14.7	-2.5 -2.0 -	15.7 15.3 -2.8	0.2 -0.2 -	I I -
		F7	BL FL PC%	144.9 150.5 3.9	-2.8 -2.7 0.4	39.0 40.5 3.8	-1.9 -2.2 -	18.6 17.9 -3.7	-0.3 -0.9 -	III IV -
		F8	BL FL PC%	124.0 133.2 7.4	-2.4 -1.9 0.4	25.6 27.8 8.6	-1.5 -1.8 -	16.6 15.7 -5.9	0.1 -0.9 -	I I -
	Only EX	F9	BL FL PC%	152.2 156.6 2.9	1.3 1.2 -0.1	42.1 51.2 21.6	1.0 1.5 -	18.2 20.9 14.9	0.6 1.4 -	III III -
		F10	BL FL PC%	130.3 137.6 5.6	-1.9 -1.6 0.2	33.2 44.4 33.7	0.2 0.7 -	19.6 23.5 19.9	1.2 2.0 -	I II -
Normal	rhGH, with EX	F11	BL FL PC%	96.1 107.0 11.3	-3.88 -2.4 2.2	13.3 14.6 9.8	-3.9 -3.6 -	14.4 12.8 -11.5	-1.0 -2.7 -	I I -
	Only rhGH	F12	BL FL PC%	153.3 155.1 1.2	-2.37 -2.6 -0.3	41.8 43.8 4.8	-2.09 -2.4 -	17.8 18.2 2.4	-0.9 -1.0 -	III IV -
	Neither rhGH or EX	F13	BL FL PC%	104 109 4.8	-2.1 -2.3 -0.1	18.6 19.4 4.3	0.5 1.1 -	17.2 16.3 -5.0	1.0 0.4 -	I I -
		F14	BL FL PC%	151.5 153.4 1.3	-1.5 -1.4 0.0	56.7 59.3 4.6	0.6 0.7 -	24.7 25.2 2.0	1.6 1.6 -	I II -

BL: baseline; FL: follow up; PC%: percentage change

Delta changes in height SDS was calculated from baseline to follow up

8.6.3.3 Densitometric changes

8.6.3.3.1 Total body

The subjects with GHD, whether they were on rhGH, EX, both or neither, showed higher positive PC% in TB- BMD,TB- BMC and TB-BA compared to the normal (Figure 8-6). However, their relative gain in TB-BMC was lower in relation to the relative gain in bone size (expressed as BMC for bone area SDS) which was noticeably declined from baseline to follow up (Figure 8-7).
There were no obvious differences in TB-BMD, BMC, and BA between those who had rhGH alone or with additional EX.

8.6.3.3.2 Lumber spine

LS- BMD, LS-BMC and LS-BMAD were modestly changed from baseline to follow up in the majority of subjects, and there was no obvious difference between those who were on rhGH alone or combined with EX (Figure 8-8).
Similar to TB-BMC for bone area SDS, LS-BMC for bone area SDS was obviously declined from baseline to follow up in subjects with GHD who were on rhGH (Figure 8-9).

When we considered the time spent in habitual physical activities and participated in sport and exercise (hours per week) as stated in Table 8-1, there were no obvious differences between those with highest physical activity (particularly patient's ID; F4, F5 who spent 3 and 6 hours a week training football respectively)), compared to those who were less active.

The individual data of TB and LS parameters are shown in Table 8-3

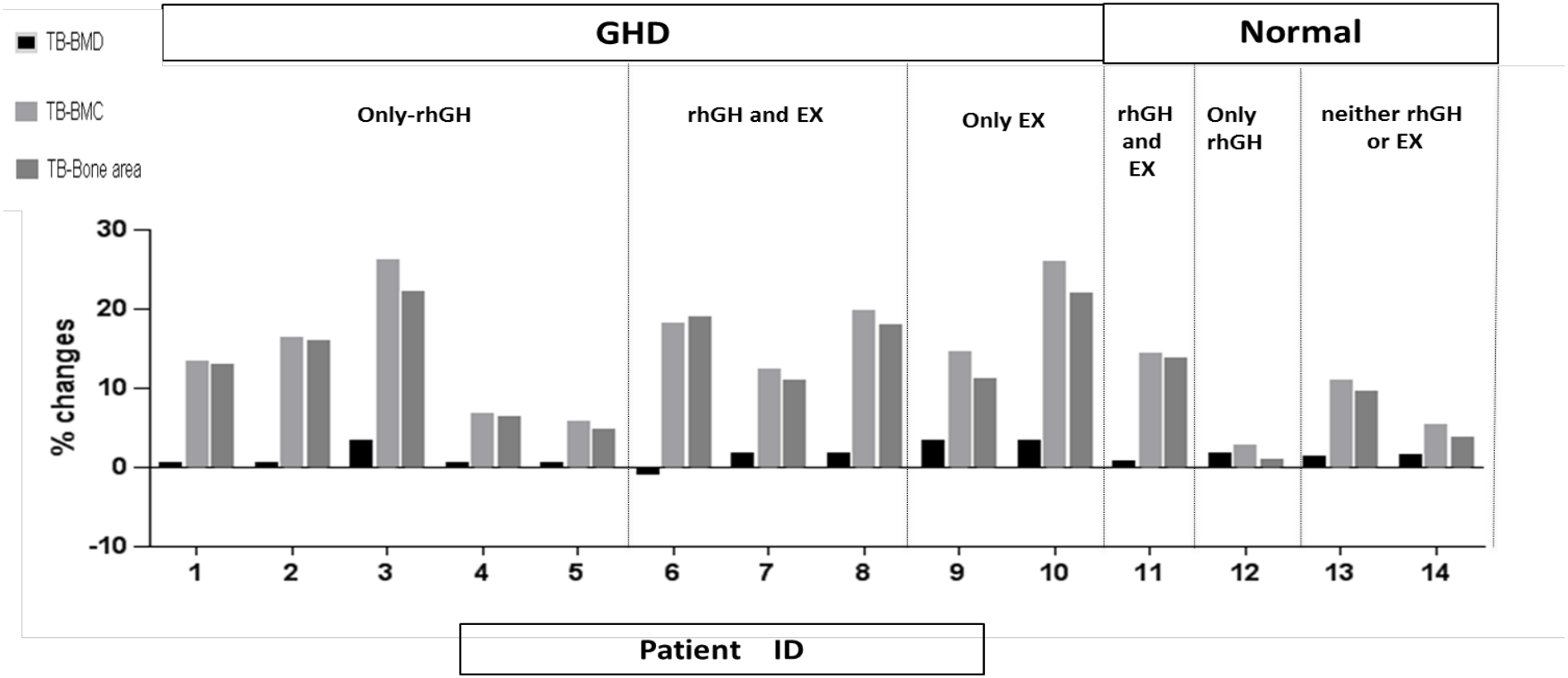

Figure 8-6 Percentage changes in total body bone parameters of the first time assessment group

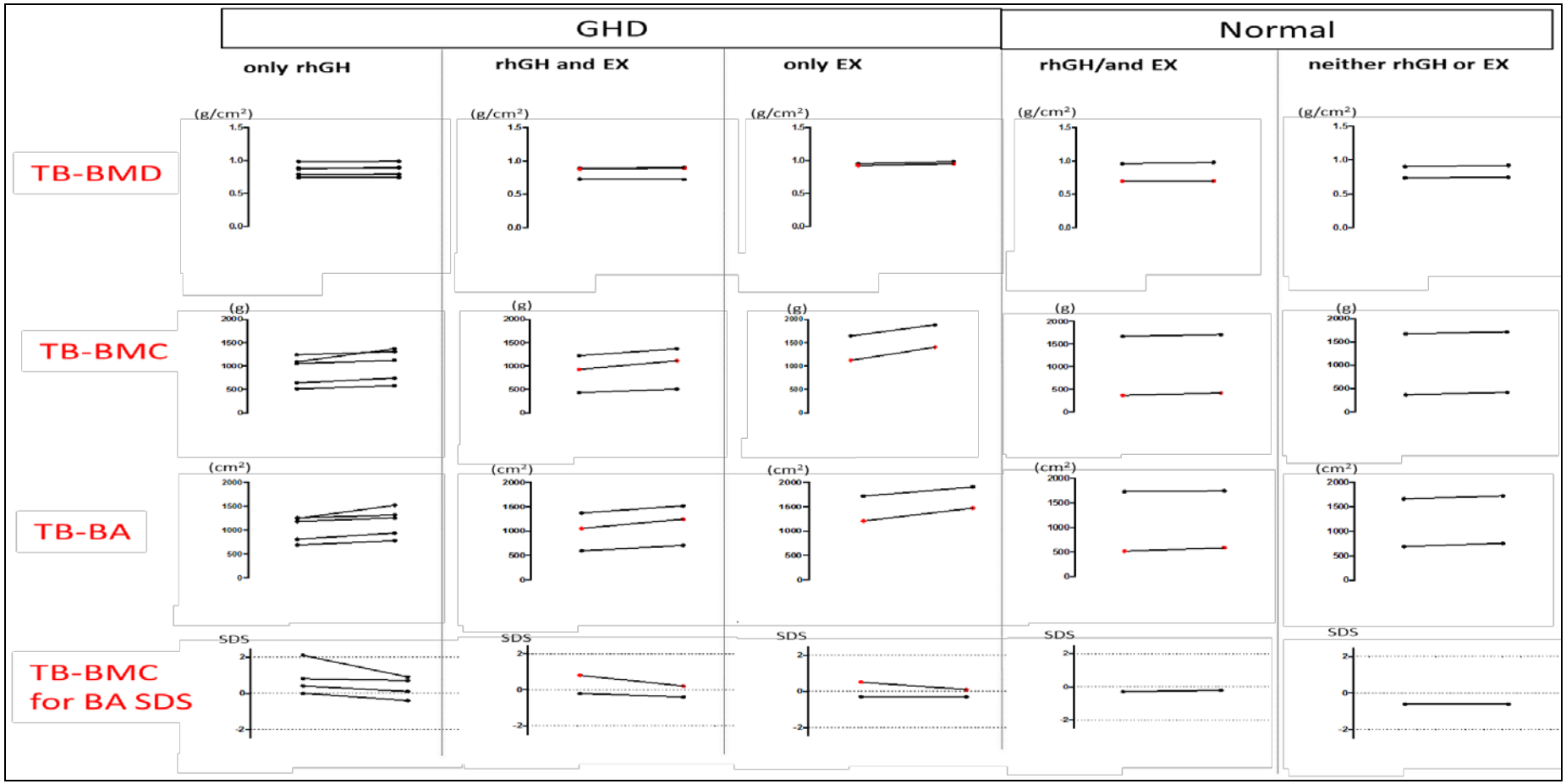

Figure 8-7 Total body bone BMD, bone area, BMC and BMC for bone area SDS at baseline and follow up of the first time assessment groups.
Red dots represented those who were on exercise but had poor compliance rate <20%

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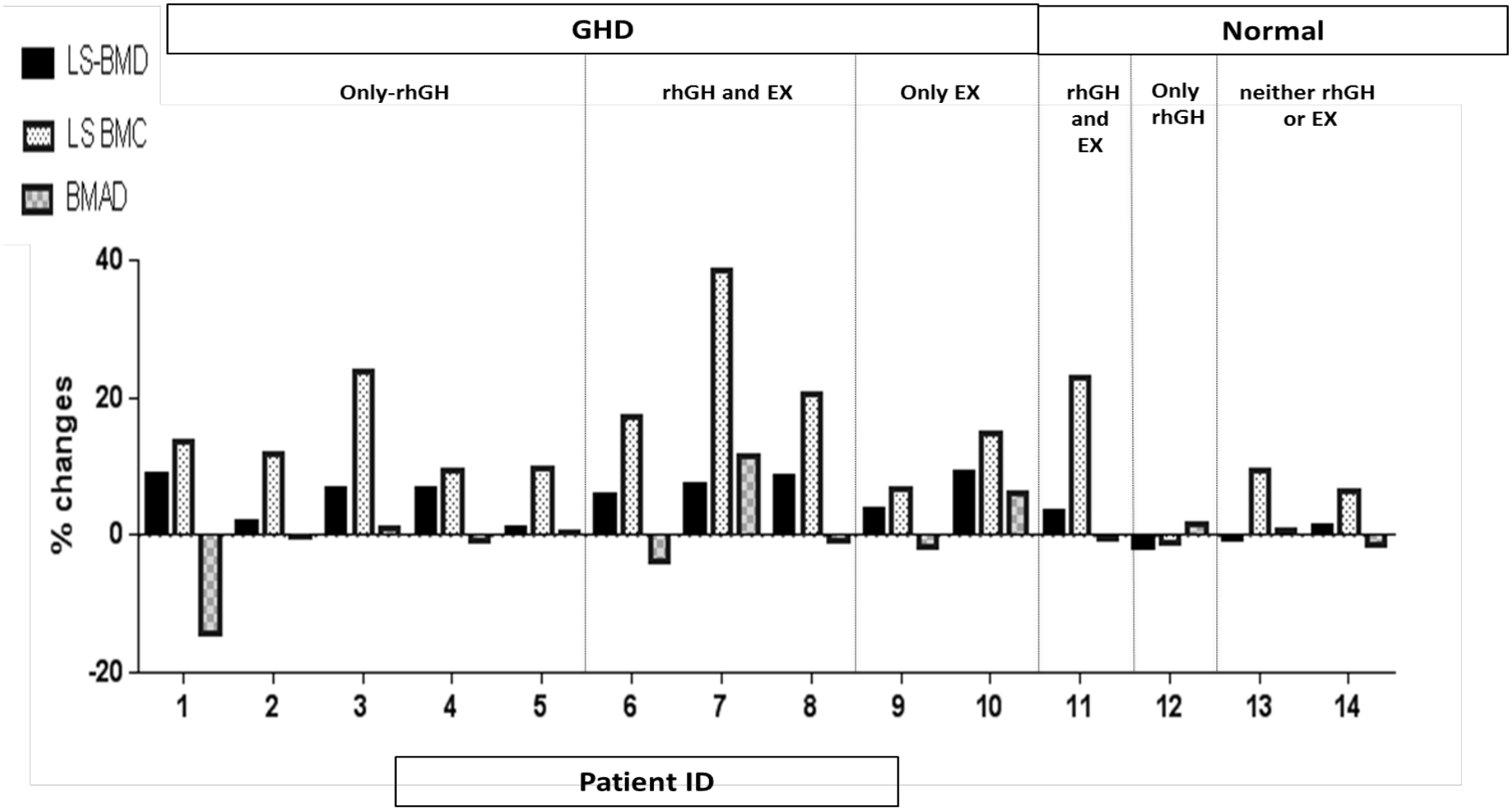

Figure 8-8 Percentage changes in lumber spine bone parameters of the first time assessment group

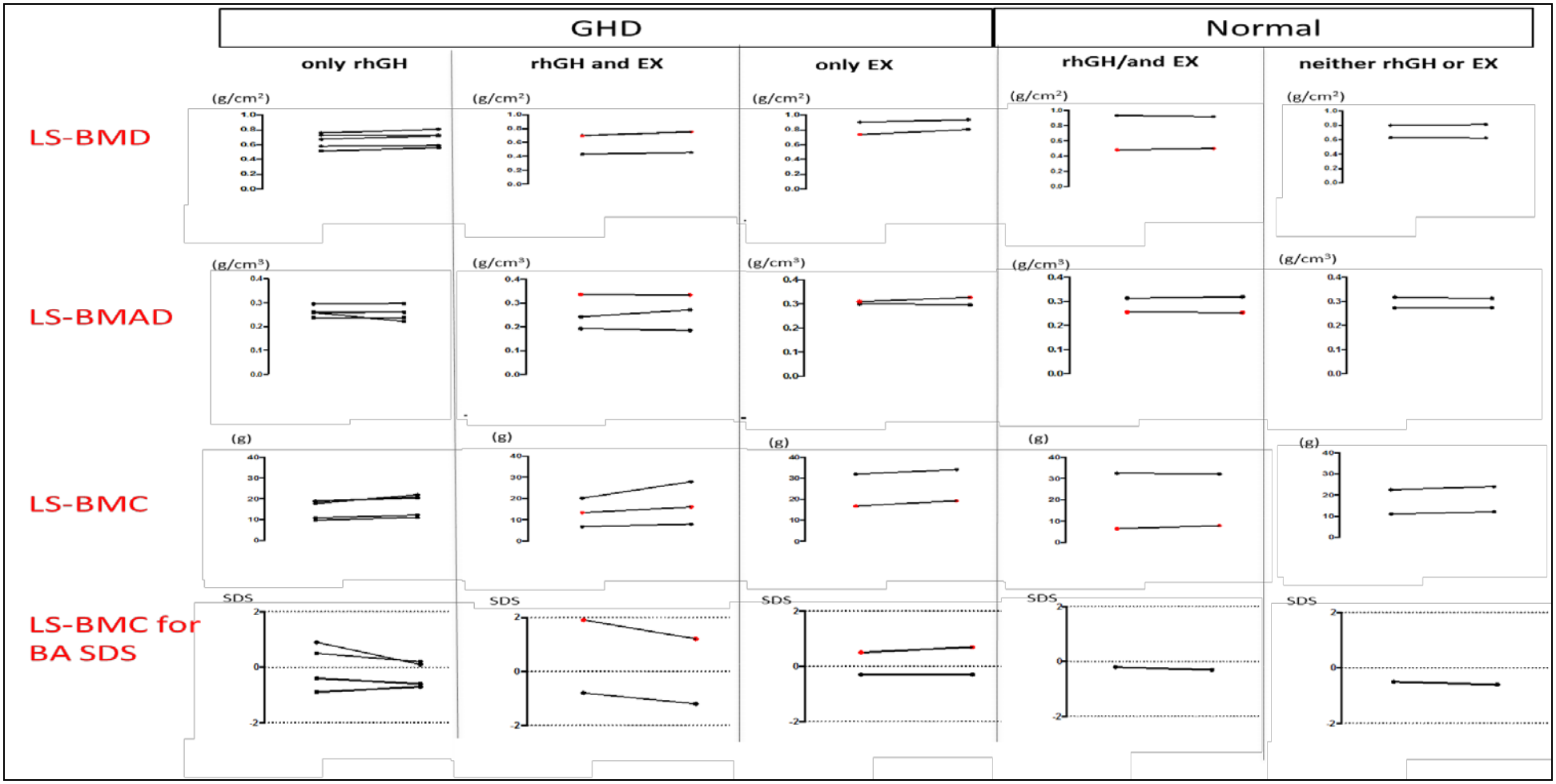

Figure 8-9 Lumber spine bone BMD, BMAD, BMC and BMC for bone area SDS at baseline and follow up of the first time assessment group.
Red dots represented those who were on exercise but had poor compliance rate <20%

Table 8-3 DXA -TB, LS bone density parameters at baseline, follow up and percentage changes in the first time assessment group.

Group		ID	Time	TB-BMD		TB-BMC		TB-BA		LS-BMD		LS-BMC		LS-BMAD		LS-BA (cm^2)
				BMD (g/cm^2)	Z score-height-age	BMC (g)	BMC/ BA SDS	BA (cm^2)	BA/H SDS	BMD (g/cm^2)	Z score-height-age	BMC (g)	BMC/ BA SDS	BMAD (g/cm^3)	BMAD SDS	
GHD	Only rhGH	F1	BL	0.747	-	515	-	689	-1.1	0.513	-	9.75	-	0.258	-2.5	19.01
			FL	0.749	-	582.5	-	778	-0.4	0.558	-	11.09	-	0.221	-1.5	19.87
			PC%	0.3	-	13.1	-	12.9	-	8.8	-	13.7	-	-14.3	-	4.5
		F2	BL	0.792	0.2	640.2	2.1	808	-0.4	0.578	-0.7	10.91	0.9	0.237	-1.1	18.86
			FL	0.794	-0.3	743.5	0.9	936	0.4	0.589	-1.0	12.2	0.1	0.237	-1.1	20.7
			PC%	0.3	-	16.1	-57.1	15.8	-	1.9	-	11.8	-	-0.3	-	9.8
		F3	BL	0.874	0.6	1087	0.0	1244	1.5	0.758	0.6	17.76	0.5	0.294	0.4	23.44
			FL	0.902	0.6	1370	-0.4	1518	2.5	0.809	0.6	21.98	0.2	0.296	0.3	27.18
			PC%	3.2	-	26.0	-	22.0	-	6.7		23.8	-	0.9	-	16
		F4	BL	0.889	0.8	1052.2	0.4	1183	-0.8	0.673	-0.5	18.77	-0.9	0.261	-0.7	27.9
			FL	0.892	0.1	1120.8	0.1	1256	-1.9	0.719	-0.5	20.53	-0.7	0.259	-0.8	28.54
			PC%	0.3	-	6.5	-	6.2	-	6.8	-	9.4	-	-0.7	-	2.3
		F5	BL	0.987	3.6	1240.2	0.8	1257	1.5	0.725	0.6	19.02	-0.4	0.258	-0.6	26.25
			FL	0.99	2.5	1310.6	0.7	1314	0.9	0.732	0	20.85	-0.6	0.259	-0.7	28.48
			PC%	0.3	-	5.7	-	4.5	-	1.0	-	9.6	-	0.4	-	8.5
	rhGH and EX	F6	BL	0.728	-	432.5	-	594	-2.4	0.43	-	6.77	-	0.193	-2.1	15.76
			FL	0.723	-	510.3	-	706	-1.5	0.455	-	7.93	-	0.185	-2.1	17.42
			PC%	-0.7	-	18.0	-	18.9	-	5.8	-	17.1	-	-3.9	-	10.5
		F7	BL	0.891	-0.2	1220.3	-0.2	1370	-1.2	0.703	-0.8	20.19	-0.8	0.242	-0.8	28.72
			FL	0.904	-0.5	1370.6	-0.4	1517	0.6	0.754	-0.8	27.95	-1.2	0.271	-2.0	37.06
			PC%	1.5	-	12.3	-	10.7	-	7.3		38.4	-	11.6	-	29.0
		F8	BL	0.88	-	929.3	0.8	1056	-0.7	0.696	-	13.34	1.9	0.336	1.5	19.17
			FL	0.893	0.6	1110.8	0.2	1245	0.1	0.755	0.2	16.06	1.2	0.334	1.5	21.27
			PC%	1.5	-	19.5	-	17.9	-	8.5	-	20.4	-	-0.9	-	11.0

	Only EX	F9	BL FL PC%	0.956 0.986 3.1	0.2 0.5 -	1641.7 1879.6 14.5	-0.3 -0.3 -	1718 1906 10.9	0.8 1.1 -	0.905 0.939 3.8	0.4 0.9	31.91 34.08 6.8	-0.3 -0.3	0.302 0.297 -1.6	0.4 0.5	35.25 36.34 3.1
		F10	BL FL PC%	0.927 0.956 3.1	1.5 1.5 -	1118.5 1406.6 25.8	0.5 0.1 -	1207 1471 21.9	-0.1 2.4 -	0.737 0.805 9.2	0.2 0.6	16.78 19.27 14.8	0.5 0.7 -	0.310 0.328 6.0	1.3 0.7 -	22.76 23.93 5.1
Normal	rhGH and EX	F11	BL FL PC%	0.699 0.703 0.6	- - -	364.5 416.2 14.2	- - -	521 592 13.6	-3.4 -3.2 -	0.481 0.497 3.3	- - -	6.35 7.81 23.0	- - -	0.256 0.254 -0.4	-0.2 -0.4 -	13.19 15.84 20.1
	Only rhGH	F12	BL FL PC%	0.962 0.978 1.7	0.5 0 -	1670.8 1712.6 2.5	-0.3 -0.2 -	1736 1751 0.9	0.8 -0.4 -	0.931 0.915 -1.7	0.9 0.0 -	32.45 32.09 -1.1	-0.2 -0.3 -	0.314 0.320 1.7	0.1 -0.5 -	34.85 35.06 0.6
	Neither rhGH or EX	F13	BL FL PC%	0.738 0.747 1.2	- - -	508.1 563 10.8	2.3 1.4 -	689 754 9.4	-0.7 -0.5 -	0.629 0.625 -0.6	- - -	11.06 12.09 9.3	- - -	0.274 0.275 0.6	-0.3 -0.5	17.6 19.35 9.9
		F14	BL FL PC%	0.906 0.919 1.4	-0.8 -0.8 -	1503.6 1583.1 5.3	-0.6 -0.6 -	1661 1722 3.7	-0.1 0.1 -	0.802 0.813 1.4	-1.2 -1.2 -	22.54 24.01 6.5	-0.5 -0.6 -	0.318 0.313 -1.4	-1.4 0.1 -	28.1 29.53 5.1

BL: baseline; FL: follow up; PC%: percentage change; TB: total body; LS: lumbar spine; H: height; BMD: bone mineral density; BMC: bone mineral content; BMAD: bone mineral apparent density; BA: bone area

8.6.3.4 Changes in body composition measured by DXA

There was a relative positive gain in LM in the majority of subjects in the group. GHD subjects who were on rhGH alone and those on a combination of rhGH with EX showed higher gain in LM compared with the other group (PC% ranging from (13.3% to 23.5%), (10.3% to 28.6%) respectively), whilst the one GHD subject with EX alone and normal subjects with neither rhGH or EX showed the lowest change in LM (PC%; 2.7%, 4.6 % and 2.5%), (Figure 8-10). Similarly, the changes in LM for height centile were obviously higher in those with GHD and on rhGH compared to others, and that was not different between those who had rhGH combined with /without EX, (Figure 8-11).

Loss of FM was observed in three of the five subjects who were GHD on only rhGH (PC%; -20%, -16%, -24%), when the other two patients (patient IDs: F1, F3) had (4.4%, 38%) increase in FM over follow-up period. Noticeably, all subjects with GHD and combined rhGH with EX showed a proportionally greater decline in FM (PC%: -44%, -15%,-28%) compared with the other. On the other hand, the two patients who were confirmed to have GHD but were only on EX (patient IDs: F9, F10) had the highest gain in FM (PC%: 111%, 80%) from the baseline to the follow up compared to the others (Figure 8-10, 8- 12). Normal subjects (patient IDs: F11, F12, F13 and F14) showed modest changes in FM regardless whether they were on rhGH, EX, or either.

A/G ratio demonstrated a slightly raised central fat distribution in three of the five GHD subjects who were on only rhGH and the two GHD subjects on only EX, whereas A/G ratio was declined in all those who were on a combination of rhGH with EX

The individual data of DXA body composition parameters in the first time assessment group are is outlined in Table 8-4

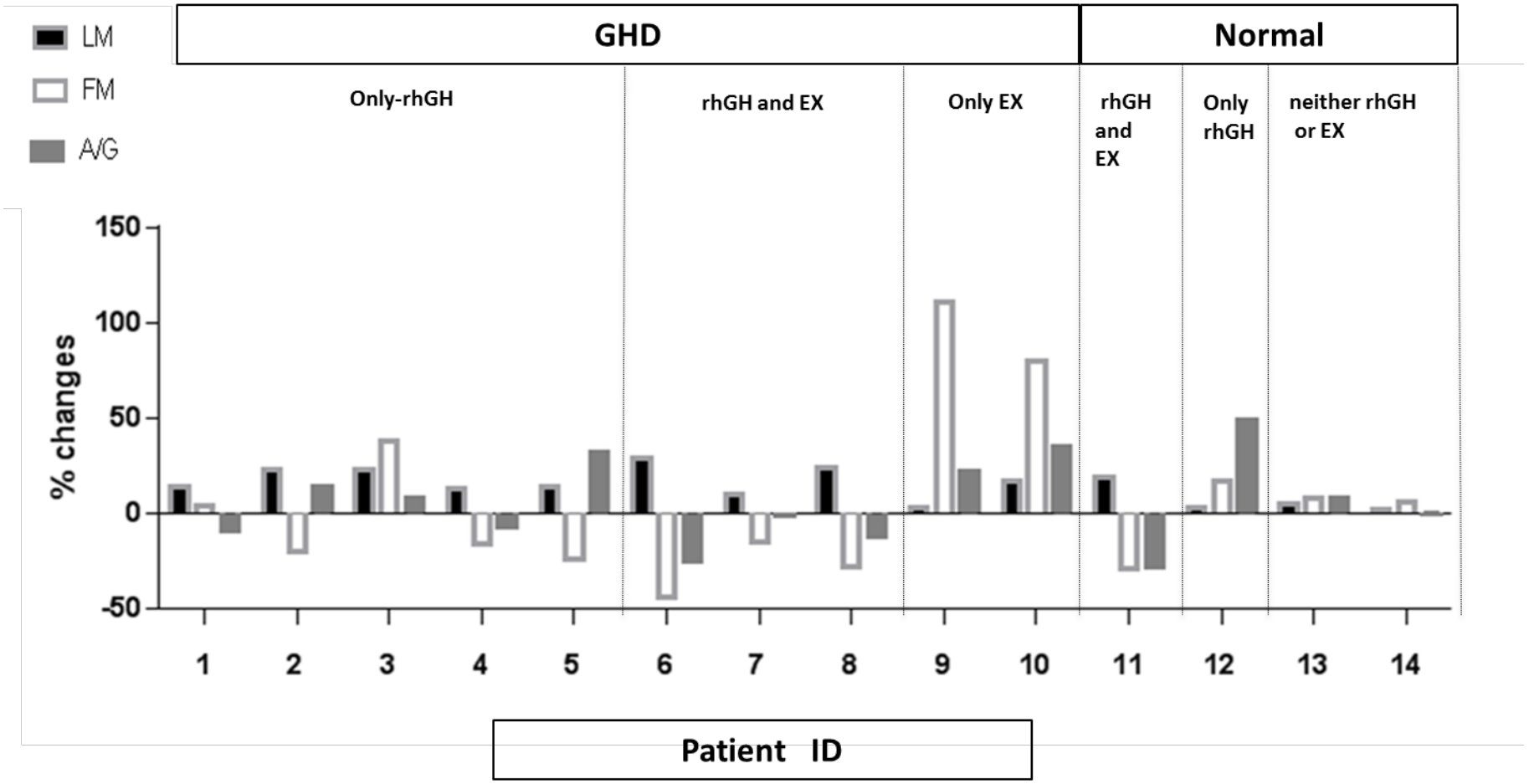

Figure 8-10 Percentage changes in body composition (LM-FM-A/G ratio) of the first time assessment group

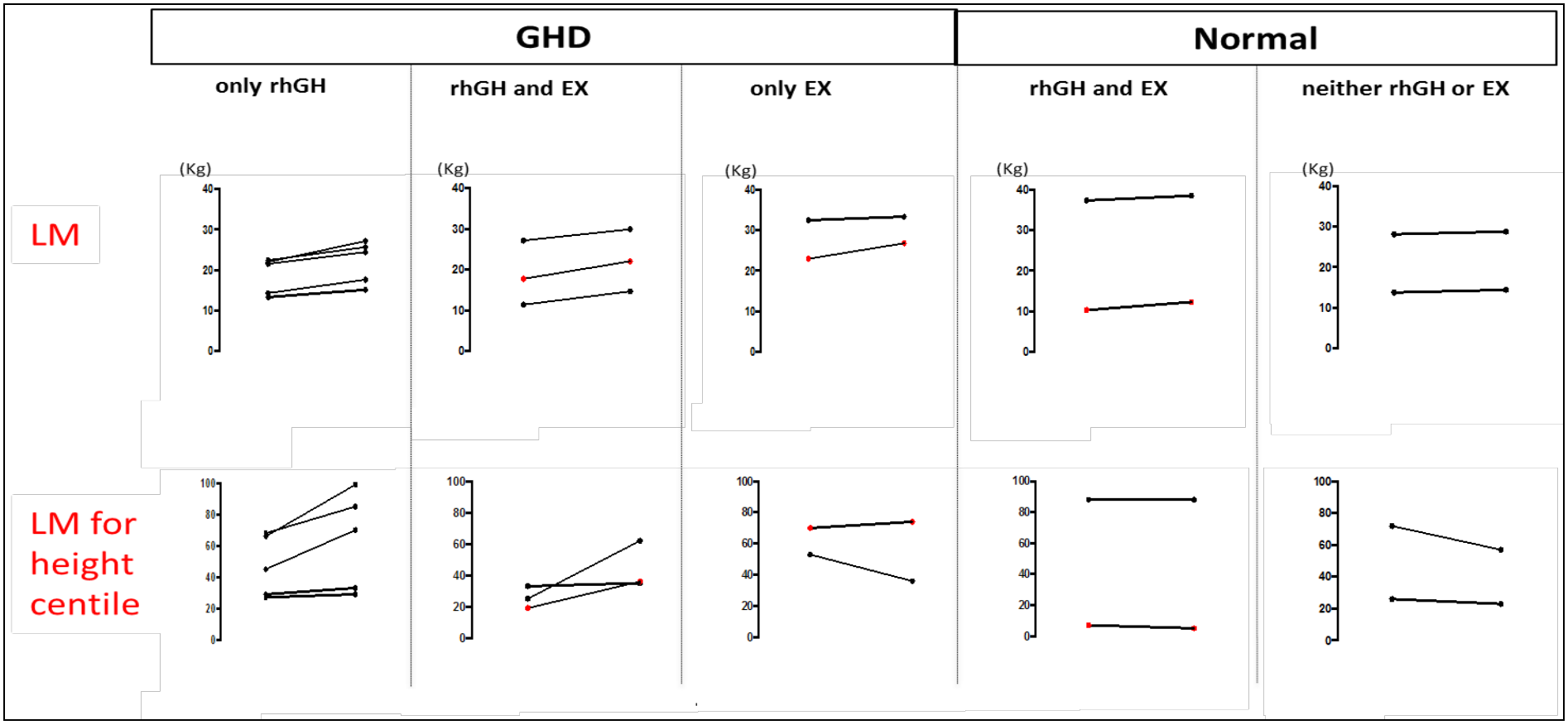

Figure 8-11 Individual data of lean mass and lean mass for height centile from baseline to follow up of the first time assessment group.
Red dots represented those who were on exercises but had poor compliance rate <20%

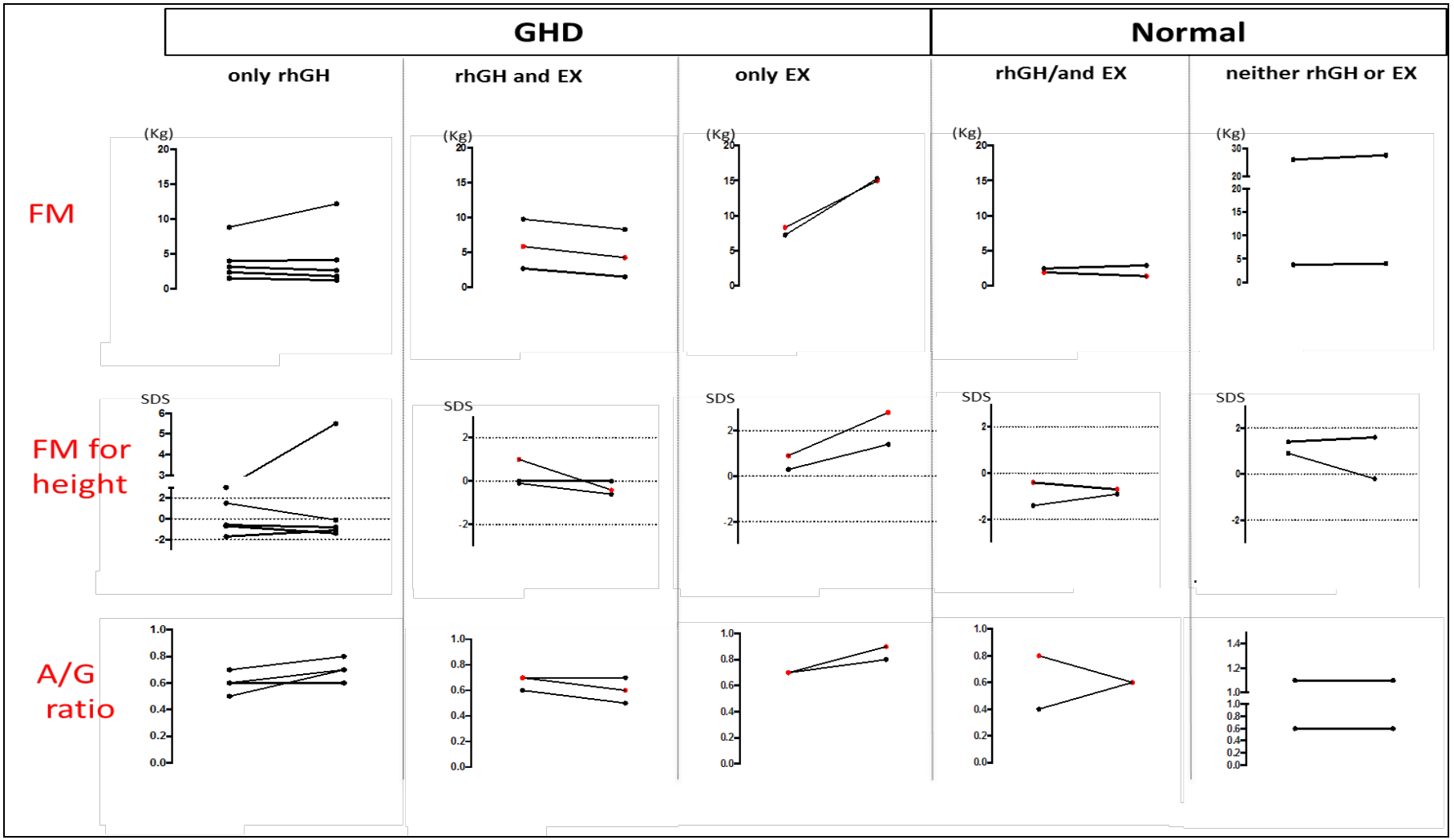

Figure 8-12 Individual data of fat mass; fat mass for height SDS, and A/G fat ratio from baseline to follow up of the first time assessment group.
Red dots represented those who were on exercises but had poor compliance rate <20

Table 8-4 DXA-body composition parameters at baseline, follow up and percentage changes in the first time assessment group.

Groups		ID	Time	LM		FM		
				LM (Kg)	LM/H centile	FM (Kg)	FM/H SDS	A/G ratio
GHD	Only rhGH	F1	BL	13.4	45	4.0	1.5	0.6
			FL	15.2	70	4.2	-0.1	0.6
			PC%	13.7	56.0	4.4	-	-9.0
		F2	BL	14.4	29	1.6	-0.6	0.6
			FL	17.7	33	1.3	-0.8	0.7
			PC%	22.8	14.0	-20.0	-	14.0
		F3	BL	22.1	66	8.9	3.0	0.7
			FL	27.2	99	12.2	5.5	0.8
			PC%	23.5	50.0	38.0	-	8.0
		F4	BL	21.6	27	3.2	-0.7	0.6
			FL	24.5	29	2.7	-1.4	0.6
			PC%	13.4	8.0	-16.0	-	-7.0
		F5	BL	22.5	68	2.4	-1.7	0.5
			FL	25.7	85	1.9	-1.1	0.7
			PC%	14.4	25.0	-24.0	-	32.0
	rhGH and EX	F6	BL	11.4	25	2.7	-0.1	0.6
			FL	14.7	62	1.5	-0.6	0.5
			PC%	28.6	148.0	-44.0	-	-25.0
		F7	BL	27.2	33	9.8	0.0	0.7
			FL	29.9	35	8.3	0.0	0.7
			PC%	10.3	6.0	-15.0	-	-1.0
		F8	BL	17.8	19	5.9	1.0	0.7
			FL	22.1	36	4.3	-0.4	0.6
			PC%	24.2	90.0	-28.0	-	-13.0
	Only EX	F9	BL	32.6	53	7.3	0.3	0.7
			FL	33.4	36	15.4	1.4	0.8
			PC%	2.7	-32.0	111.0	-	21.9
		F10	BL	23.0	70	8.4	0.9	0.7
			FL	26.9	74	14.9	2.8	0.9
			PC%	16.9	5.7	80.0	-	34.9
Normal	rhGH and EX	F11	BL	10.3	7	1.9	-0.4	0.8
			FL	12.3	5	1.4	-0.7	0.6
			PC%	19.4	-28.6	-28.5	-	-27.9
	Only rhGH	F12	BL	37.5	88	2.5	-1.4	0.4
			FL	38.6	88	2.9	-0.9	0.6
			PC%	3.1	0.0	17.5	-	48.6
	Neither rhGH or EX	F13	BL	13.8	72	3.7	0.9	0.6
			FL	14.4	57	3.8	-0.2	0.6
			PC%	4.6	-20.0	8.1	-	7.9
		F14	BL	28.2	26	25.9	1.4	1.1
			FL	28.8	23	27.7	1.6	1.1
			PC%	2.5	-11.0	6.3	-	-0.1

BL: baseline; FL: follow up; PC%: percentage change; LM: lean mass; FM: fat mass; FM/H SDS; fat mass for height SDS; A/G: Android/Gynoid fat ratio

8.6.3.5 Tibia bone and muscle parameters

Of the 14, 11 had their pQCT scans done at baseline and follow up. Two subjects' (patient IDs: F4, F6) geometric data at the 38% site were excluded for movement error, Table 8-5.

From the available data, there was variability in the cortical thickness, periosteal circumferences and endo-osteal circumferences across the group, Figure 8-13. Cortical thickness and periosteal circumferences tended to expand in GHD on rhGH with/ without EX, without obvious change in endo-osteal circumferences. The two GHD on only EX (patient IDs: F 9, F10) showed small reduced in cortical thickness and enlarged in periosteal and endo-osteal circumferences. These measurements were not obviously changes in the two normal subjects with neither rhGH or EX (patient IDs: F13, F14).

Cortical density tended to fall in all GHD subjects receiving rhGH, when trabecular density increased only in three subjects (patient IDs: F4, F6, F8). Total density remained unchanged in the majority of subjects.

Muscle-CSA tended to enlarge only in subjects who received rhGH, when those with GHD on only EX (patient IDs: F9, F10) had lower muscle CSA and height fat CSA on follow up than their baseline data, Table 8-6.

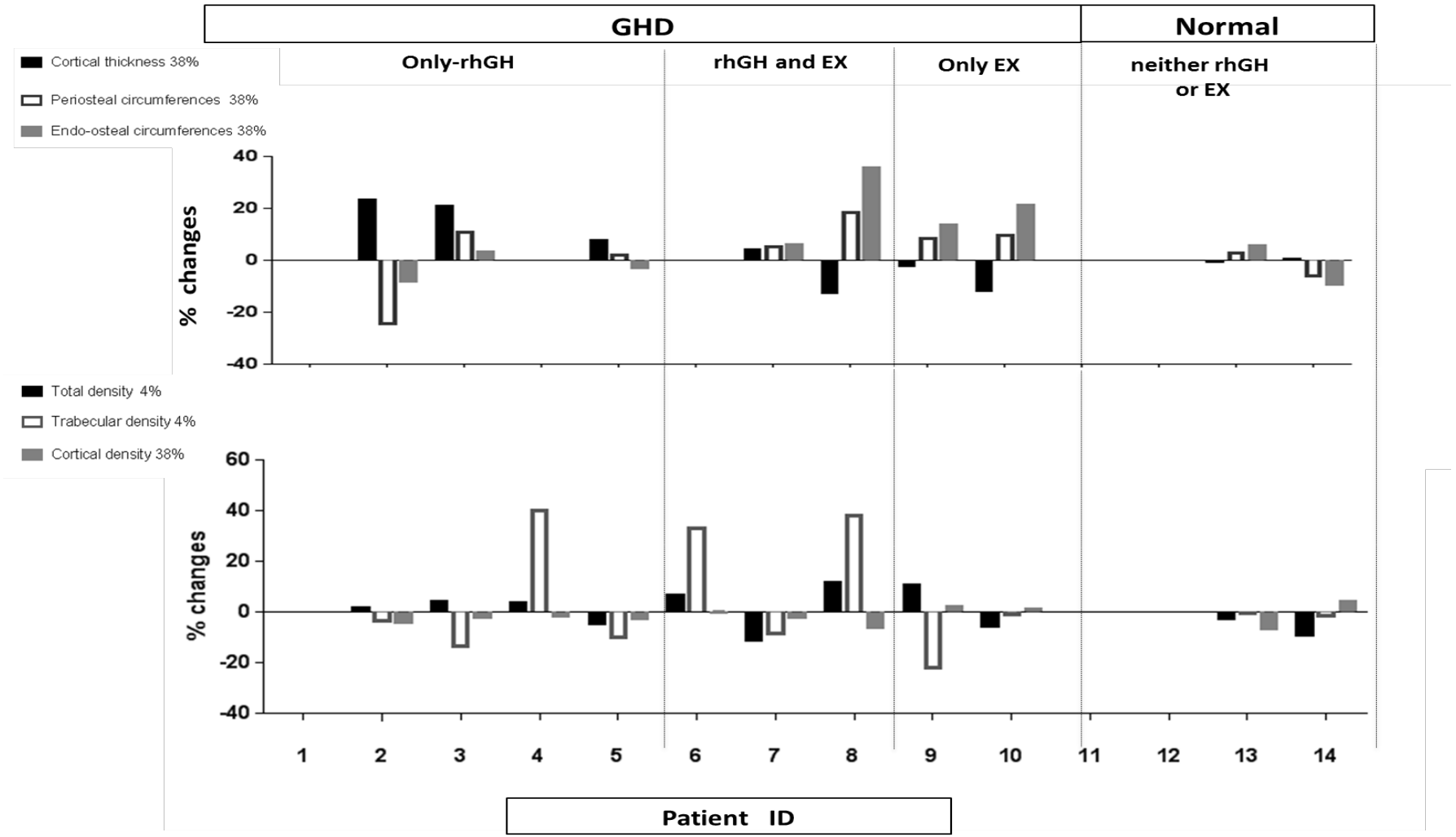

Figure 8-13 Percentage changes in tibia pQCT parameters from baseline to follow up of the first time assessment group

Table 8-5Tibia pQCT parameters (4% and 38% sites) at baseline, follow up and percentage changes of the first time assessment group

		Site		38%		38%		38%		4%		38%		4%	38%
		Measurement		CTh		PC		EC		TrvBMD		CvBMD		TvBMD	SSI
		ID	Time	(mm)	Height - z-score	(mm)	Height - z-score	(mm)	Height -z-score	mg/cm 3	Age -Z score	mg/cm^3	Age -Z score	mg/cm^3	
GHD	Only rhGH	F1	BL	-	-	-	-	-	-	-	-	-	-	-	-
			FL	-	-	-	-	-	-	-	-	-	-	-	-
			PC%	-	-	-	-	-	-	-	-	-	-	-	-
		F2	BL	2.3	0.8	48.5	-0.1	33.8	-0.5	182.1	-0.6	1061.7	1.2	275.3	328.1
			FL	2.9	0.5	36.6	-1.7	31.1	-1.0	176.0	-0.9	1018.5	-0.2	279.9	77.1
			PC%	23.3	-	24.5	-	-8.0	-	-3.4	-	-4.1	-	1.7	-76
		F3	BL	3.6	0.7	54.3	-0.6	31.7	-1.0	245.6	1.1	1024.9	-0.1	318.9	538.2
			FL	4.3	1.0	60.1	-0.1	32.8	-1.2	212.8	0.1	1002.3	-0.9	331.1	792.9
			PC%	20.9	-	10.7	-	3.4	-	-13.4	-	-2.2	-	3.8	47.3
		F4	BL	-	-	-	-	-	-	184.5	-0.9	1116.0	1.8	284.2	696.4
			FL	-	-	-	-	-	-	258.4	1.1	1102.0	1.3	294.0	753.9
			PC%	-	-	-	-	-	-	40.0	-	-1.3	-	3.5	8.3
		F5	BL	4.2	1.3	57.9	0.3	31.7	-1.0	258.0	1.4	1113.8	2.1	380.8	743.8
			FL	4.5	1.4	59.8	0.2	30.9	-1.4	232.0	0.7	1084.1	1.3	363.6	825.6
			PC%	7.6		2.0	-	-2.7		-10.0	-	-2.7	-	-4.5	11
	rhGH and EX	F6	BL	1.7	3.6	43.1	-0.4	33.0	-1.6	144.2	-2.0	1022.3	-0.1	241.8	225.1
			FL	-	-	-	-	-	-	192.1	-0.2	1021.5	0.0	257.3	232.0
			PC%	-	-	-	-	-	-	33.2		-0.1		6.4	3.0
		F7	BL	3.5	-0.2	63.8	0.0	41.6	0.3	238.8	0.2	1086.6	0.2	321.8	986.7
			FL	3.7	-0.2	67.2	0.3	44.2	0.5	218.1	-0.5	1064.0	-0.7	286.6	984.5
			PC%	3.8	-	5.3	-	6.2	-	-8.7	-	-2.1	-	-10.9	-0.2

		F8	BL FL PC%	3.1 2.7 -12.3	0.3 -0.9 -	53.9 63.8 18.4	-0.2 1.3 -	34.6 46.9 35.6	-0.2 1.9 -	281.4 388.5 38.1	2.0 3.9 -	1092.4 1025.4 -6.1	1.7 -0.3 -	341.0 380.7 11.6	522.1 510.4 -2.2
	Only EX	F9	BL FL PC%	4.0 3.9 -1.9	0.1 -0.1 -	75.6 82.0 8.4	2.0 2.8 -	50.6 57.4 13.4	1.6 2.4 -	221.4 172.9 -21.9	0.4 -1.3 -	1067.3 1089.0 2.0	0.9 1.3	281.9 311.5 10.5	1267.1 1402.7 10.7
		F10	BL FL PC%	3.6 3.2 -11.5	0.5 -0.4 -	64.1 70.2 9.6	1.7 2.3 -	41.5 50.2 21.0	1.1 2.3 -	278.4 276.1 -0.8	1.8 1.6 -	1085.1 1094.6 0.9	1.4 1.3 -	321.4 304.3 -5.3	906.3 889.1 -1.9
Normal	rhGH and EX	F11	BL FL PC%	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -
	Only rhGH	F12	BL FL PC%	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -
	Neither rhGH or EX	F13	BL FL PC%	2.4 2.0 -17.6	0.6 -1.5 -	47.4 50.2 6.0	-0.1 0.2 -	32.3 37.8 17.0	-0.2 0.8 -	208.2 207.7 -0.3	0.4 0.4 -	1046.2 980.1 -6.3	1.1 -1.0 -	303.9 296.1 -2.6	305.1 329.3 7.9
		F14	BL FL PC%	3.3 3.9 15.9	-1.0 0.0 -	72.6 66.6 -8.3	1.6 0.1 -	51.6 42.2 -18.1	1.9 0.2 -	172.3 169.7 -1.5	-1.4 -1.6 -	1049.9 1089.7 3.8	-2.0 -1.1 -	242.3 221.0 -8.8	1067.7 1085.7 1.7

BL: baseline; FL: follow up; PC%: percentage change; CTh: Cortical thickness; PC: Periosteal circumference; EC: Endosteal circumference; TvBMD: Total density; TrvBMD: Trabecular density; CvBMD: Cortical density; SSI: Strength strain index

Table 8-6 Tibia pQCT parameters at 66% site at baseline, follow up and percentage changes in the first time assessment group.

Group		Site		66%			
		ID	Time	Cortical CSA (mm^2)	Muscle CSA (mm^2)	Fat CSA (mm^2)	Bone/Muscle ratio
GHD	Only rhGH	F1	BL FL PC%	- - -	- - -	- - -	- - -
		F2	BL FL PC%	121.0 140.3 15.9	2170.5 2724.5 25.5	789.5 707.0 -10.4	5.6 5.2 -7.5
		F3	BL FL PC%	191.3 232.8 21.7	3799.3 4167.8 9.7	1842.8 2245.3 21.8	5.0 5.5 9.3
		F4	BL FL PC%	190.5 209.8 10.1	3488.0 3625.0 3.9	1006.5 949.5 -5.7	5.5 5.8 6.0
		F5	BL FL PC%	238.0 256.3 7.7	3970.0 4670.3 17.6	910.5 661.3 -27.4	6.0 5.5 -8.3
	rhGH and EX	F6	BL FL PC%	77.3 53.5 -30.7	1989.8 2253.8 13.3	1031.0 826.8 -19.8	3.9 2.4 -38.9
		F7	BL FL PC%	219.0 253.5 15.8	5016.5 5005.8 -0.2	2504.3 2016.3 -19.5	4.4 5.1 15.8
		F8	BL FL PC%	163.3 201.0 23.1	2940.3 3538.3 20.3	2109.8 1359.0 -35.6	5.6 5.7 2.3
	Only EX	F9	BL FL PC%	292.8 284.0 -3.0	4889.3 4930.8 0.8	1618.0 2284.3 41.2	6.0 5.8 -3.9
		F10	BL FL PC%	238.5 299.3 25.5	4070.0 3674.5 -9.7	2134.3 2969.5 39.0`	5.9 8.1 38.1
Normal	rhGH and EX	F11	BL FL PC%	- - -	- - -	- - -	- - -
	Only rhGH	F12	BL FL PC%	- - -	- - -	- - -	- - -
	Neither rhGH or EX	F13	BL FL PC%	71.3 89.8 26.0	2368.5 2312.0 -2.4	1118.0 1110.1 -0.7	3.0 3.9 28.9
		F14	BL FL PC%	238.8 248.5 4.1	4633.3 4505.0 -2.8	2964.8 2670.0 -9.9	5.2 5.5 7.2

BL: baseline; FL: follow up; PC%: percentage change

8.6.3.6 Changes in bone profiles and metabolism

Only 9/14 subjects had blood samples available for both baseline and follow up. Overall, there were no apparent changes from baseline to follow up in bone profiles (Ca-Po4-Mg) in any of these groups (GHD, normal), all remained within the normal range. PTH and 25OH vit-D remained steady during the study period in those who received rhGH in either groups, but tended to rise in those who were not receiving rhGH, (Table 8-7).

The changes in bone turnover markers (BAP-OC-CTX) were higher in GHD receiving rhGH alone or combined with EX than others.

Table 8-7 Bone minerals, bone biomarkers at baseline, follow up and percentage changes in the first time assessment group

Groups		ID	Time	Bone mineralisation					Bone biomarkers		
				Ca mmol/l	Po4 mmol/l	Mg mmol/l	PTH pmol/l	Vit-D nmol/l	BAP µg/l	OC ng/ml	CTX ng/ml
GHD	Only rhGH	F1	BL FL PC%	2.2 2.3 4.5	1.38 1.2 -13.0	0.8 - -	2.9 3.8 30.0	55 39 -30.0	62.1 83.8 35.0	48.8 103.3 112.0	1.8 1.8 2.0
		F2	BL FL %	2.36 - -	1.41 - -	0.8 - -	4 - -	102 - -	110.7 - -	58.1 - -	2.1 - -
		F3	BL FL PC%	2.45 2.5 2.0	1.3 1.5 15.0	1.0 0.8 -18.0	3.7 2.8 -25.0	64 48 -25.0	138.4 85.4 -38.0	29.3 38.9 33.0	0.8 1.4 73.0
		F4	BL FL PC%	2.46 2.58 5.0	1.26 1.64 30.0	. 0.8 -	- 3.2 -	- 34 -	- - -	43.7 51.7 18.0	1.1 1.9 68.0
		F5	BL FL PC%	2.34 2.6 11.0	1.31 1.31 0.0	0.8 0.8 0.0	2.3 2.9 26.0	68 68 0.3	89.5 135.7 51.0	31.6 - -	1.6 2.1 30.0
	rhGH and EX	F6	BL FL PC%	2.32 2.4 4.0	1.61 1.89 18.0	0.8 0.8 0.0	4.2 4.7 10.0	56 56 0.5	24.3 17.3 -29.0	39.2 44.6 14.0	3.4 2.0 -40.0
		F7	BL FL PC%	2.4 . -	1.58 - -	0.9 0.8 0.0	5.8 - -	23 - -	136.8 90.3 -34.0	75.3 98.3 31.0	3.9 1.6 -60.0
		F8	BL FL PC%	2.51 2.4 -5.0	1.39 1.35 -3.0	0.8 0.8 0.0	4.0 3.6 -9.0	39 42 8.0	67.0 75.3 12.0	65.7 94.7 44.0	1.9 0.7 -64.0
	Only EX	F9	BL FL PC%	2.32 2.5 8.0	1.24 1.4 13.0	0.78 0.93 19.0	3.9 4.6 18.0	64 51 -20.0	99.0 125.3 27.0	71.7 83.6 17.0	2.0 1.9 -4.0
		F10	BL FL PC%	2.4 - -	1.4 - -	0.66 - -	5.9 - -	29 - -	110.0 - -	77.7 - -	2.4 - -
Normal	rhGH and EX	F11	BL FL PC%	2.2 - -	1.38 - -	0.8 - -	8.7 - -	35 - -	76.7 - -	42.1 - -	1.3 - -
	Only rhGH	F12	BL FL PC%	2.33 - -	1.2 - -	0.75 - -	1.4 - -	36.0 - -	50.0 - -	34.4 - -	1.3 - -
	Neither rhGH or EX	F13	BL FL PC%	2.2 2.3 3	1.46 1.4 -4.0	0.93 0.94 1.1	6.7 5.5 -18.0	20 27 35.0	51.4 52.1 1.5	69.7 31.8 -54.0	1.9 2.06.1
		F14	BL FL PC%	2.4 2.6 8.0	1.0 1.4 44.0	0.89 0.89 0.0	3.6 6.7 88.0	27 36 33.0	181.0 105.3 -41.0	51.6 76.5 48.0	1.9 0.9 -52.0

BL: baseline; FL: follow up; PC%: percentage change; Ca: calcium; PO4: phosphate; mg: magnisum; PTH: parathyroid hormone; 25 (OH) Vit-D: 25 hydroxy vitamin D; BAP: bone-specific alkaline phosphatase; OC: osteocalcine; CTX: cross linked C-telopeptide of type I collagen

8.6.3.7 Changes in metabolic profiles

From the available data, there were no noticeable changes in any of the metabolic parameters from baseline to follow up across groups over the study duration as seen in Table 8-8. However, the GHD patient (patient ID; F9) with only EX had higher total cholesterol, LDL and TG levels on follow up than the baseline. Similarly, adipokines measurements (leptin- adiponectin- resistin) were noticeably variable among the groups.

There was also an increase in fasting glucose levels, but a decrease in insulin and HOMA-IR values in the majority of the groups over the study period. Only one patient (F1- GHD on rhGH alone) had a rather higher F-insulin and HOMA-IR (PC%: 141%, 100%, respectively).

Table 8-8 Metabolic profiles at baseline, follow up and percentage changes in the first time assessment group.

	Group	ID	Time	Lipid profiles					Adipokines			Glucose homeostasis		
				T-Chol mmol/l	HDL mmol/l	LDL mmol/l	TG mmol/l	FFA mmol/l	Leptin ng/ml	Adiponectin ng/l	Resistin ng/ml	F-Glucose mmol/l	F-Insulin uIU/ml	HOMA-IR
GHD	Only rhGH	F1	BL FL PC%	3.7 3.3 -11.0	- 0.9 -	- 3.7 -	0.5 0.6 20.0	0.44 0.54 23.0	7.0 3.7 -47.0	25.9 11.0 -58.0	4.3 4.3 1.4	4.7 3.8 -19.0.	7.3 17.6 141.0	1.5 3.0 100.0
		F2	BL FL PC%	2.8 - -	1.1 - -	2.5 - -	0.7 - -	0.7 - -	1.1 - -	22.2 - -	4.8 - -	3.7 - -	8.01 - -	1.3 - -
		F3	BL FL PC%	3.5 2.9 -18.0	1.24 1.0 -20.0	2.8 2.9 3.6	1.` 1.2 16.0	0.92 0.74 -20.0	12.9 5.4 -58.0	8.0 11.6 45.0	2.4 2.6 5.9	4.6 4.9 7.0	26.2 16.3 -38.0	5.7 3.3 -41.0
		F4	BL FL PC%	- 3.7 -	- 1.9 -	- 1.9 -	- 0.8 -	- 0.6 -	1.7 - -	7.2 - -	3.3 - -	4.7 5.9 26.0	9.2 - -	2.4 - -
		F5	BL FL PC%	3.87 3.8 -1.8	1.2 1.3 4.0	3.1 2.9 -6.3	0.6 0.6 9.0	0.70 0.33 -52.0	1.8 1.0 -47.0	10.1 6.8 -33.0	4.2 5.4 29.0	4.1 4.6 12.0	4.2 - -	0.9 - -
	rhGH and EX	F6	BL FL PC%	4.3 4.4 2.3	1.6 1.4 -13.0	2.7 3.1	0.7 1.5 114.0	1.08 0.76 -30.0	1.4 2.8 101.0	10.1 19.9 97.0	3.0 3.8 25.0	3.8 5.0 31.0	5.8 6.25 8.0	1.3 1.1 -18.0
		F7	BL FL PC%	5.2 - -	1.81 - -	2.9 - -	1.5 - -	0.54 0.25 -54.0	11.6 2.8 -75.0	9.3 9.8 6.2	4.0 2.9 -26.0	4.5 - -	25.01 13.6 -45.0	5.0 2.5 -50.0
		F8	BL FL PC%	3.93 4.35 11.0	1.4 1.86 23.0	2.8 - -	1.3 0.4 -66.0	0.59 0.67 13.6	30.5 3.6 -88.0	7.6 20.0 165.0	7.0 5.1 -28.0	4.7 - -	5.8 6.7 17.0	1.2 - -

	Only EX	F9	BL FL PC%	3.8 4.7 24.0	1.5 1.8 20.0	2.5 2.6 4.0	0.6 0.9 50.0	0.86 0.21 -75.0	11.3 3.5 -70.0	8.8 5.9 -34.0	3.3 4.2 25.0	4.7 4.6 -2.0	27.4 19.2 -30.0	5.6 4.0 -28.0
		F10	BL FL PC%	3.63 - -	1.55 - -	2.3 - -	0.6 - -	0.31 - -	15.0 - -	12.1 - -	3.2 - -	4.6 - -	15.1 - -	3.1 - -
Normal	rhGH and EX	F11	BL FL PC%	3.3 - -	1.04 - -	3.2 - -	0.6 - -	1.56 - -	2.2 - -	20.6 - -	3.1 - -	3.9 - -	3.5 - -	0.6 - -
	Only rhGH	F12	BL FL PC%	4.1 - -	1.32 - -	3.1 - -	0.68 - -	1.37 - -	0.9 - -	16.0 - -	3.0 - -	3.7 - -	10.8 - -	1.8 - -
	Neither rhGH or EX	F13	BL FL PC%	4.1 4.4 7.0	0.9 0.8 -6.0	4.8 5.5 15.0	0.62 0.6 -3.0	0.6 0.8 38.0	1.8 1.6 -13.0	16.1 15.6 -3.0	4.1 2.4 -40.0	4.4 4.9 0.5	14.2 1.6 -12.0	3.1 0.3 -88.0
		F14	BL FL PC%	4.0 4.3 8.0	1.4 1.0 -27.0	- 4.3 -	1.4 2.6 85.0	0.7 0.7 0.4	28.2 41.7 48.0	5.9 8.0 37.0	5.2 9.4 81.0	4.0 4.9 0.9	- - -	- - -

BL: baseline; FL: follow up; PC%: percentage change; T-Chol: total Cholesterol; LDL: low density lipoprotein cholesterol; HDL: high density lipoprotein cholesterol; TG; triglyceride; FFA: free fatty acid; F: fasting; HOMA-IR: homeostasis model assessment insulin resistance index

8.6.3.8 Changes in quality of life measures (SF-36)

Thirteen of the fourteen subjects had a complete QoL assessment from baseline to follow up. Three out of the five children with GHD on rhGH alone showed improvement in overall total SF-36 scores from baseline to follow up, where all those with combined rhGH and EX showed rather slight diminished total scores, Figure 8-14. Among the SF-36 subscales (Table 8-9) physical functioning, social functioning, emotional role functioning and mental health scales did not change over the study period in the whole cohort. Of notice, scores of emotional wellbeing in children on rhGH were higher at baseline than those who had no rhGH, but became lower on follow up. Similarly, energy/fatigue subscale scores appear to be diminished in children with GHD in four of the five who were on rhGH alone and all three GHD subjects who were on combined rhGH and EX. Inversely, this scale seems to improve in those who have GHD on only EX.

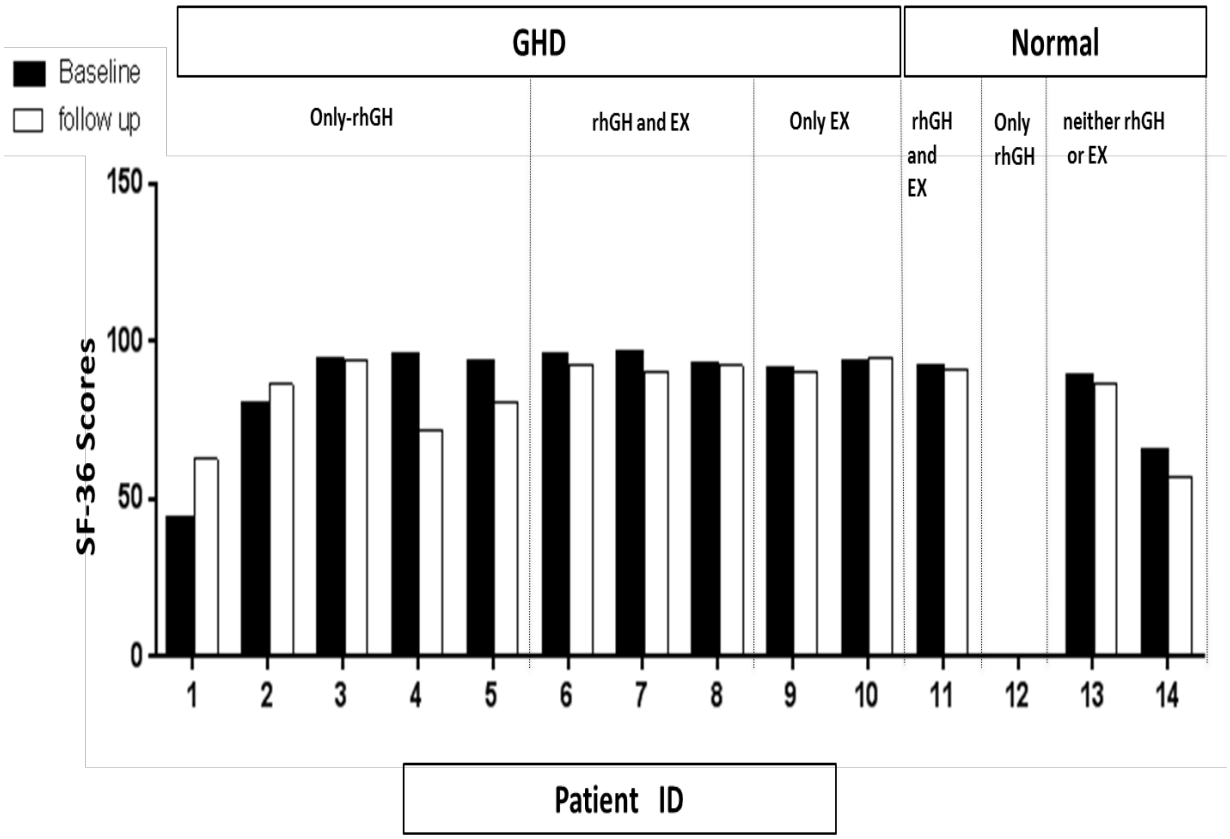

Figure 8-14 Individual QoL-SF-36 at baseline and follow up of the first time assessment group.
(Higher scores reflect better QoL)

Table 8-9 SF-36 scores at baseline, follow up and absolute changes in the first time assessment group

Group		ID	Time	Total	Physical components					Mental components					Previous year
					Total-PC	P.F	R.P	Pain	G.H	Total-MC	R.E	E/F	E.W.B	S.F	
GHD	Only rhGH	F1	BL	44	23	10	50	55	40	61	100	35	92	25	50
			FL	63	34	70	50	48	40	68	100	60	76	37.5	75
			PC%	43	45	600	0	-13	0	13	0	71	-17	50	50
		F2	BL	80	32	85	25	100	95	85	100	65	88	87.5	50
			FL	87	47	85	100	57.5	100	86	100	60	84	100	100
			PC%	8	44	0	300	-42	5	1	0	-7	-4	14	100
		F3	BL	93.8	37	100	100	60	90	94	100	80	96	100	50
			FL	94.0	39	100	100	100	90	90	100	65	96	100	50
			PC%	0.2	5	0	0	66	0	-4	0	-18	0	0	0
		F4	BL	96	36	100	100	80	95	96	100	100	84	100	50
			FL	72.1	23	80	100	57.5	45	71	100	55	68	62.5	25
			PC%	-25	-35	-20	0	-28	-52	-25	0	-45	-19	-37	-50
		F5	BL	93	36	100	100	100	75	93	100	75	96	100	50
			FL	81	26	95	100	67.5	51.2	78	100	50	88	75	25
			PC%	-13	-26	-5	0	-32	-31	-15	0	-33	-8	-25	-50
	rhGH and EX	F6	BL	96	36	100	100	100	90	94	100	85	92	100	50
			FL	93	43	100	100	100	90	88	100	65	88	100	75
			PC%	-3	19	0	0	0	0	-6	0	-23	-4	0	50
		F7	BL	96	49	100	100	100	100	92	100	75	92	100	100
			FL	91	42	100	100	100	75	88	100	65	88	100	75
			PC%	-6	-15	0	0	0	-25	-4	0	-13	-4	0	-25
		F8	BL	93	36	95	100	100	88	91	100	80	88	100	50
			FL	92	36	100	100	100	95	95	100	65	80	100	50
			PC%	-0.6	-0.8	5	0	0	9	4	0	-18	-9	0	0

	Only EX	F9	BL FL PC%	91 90 -0.1	35 36 1.2	100 85 -15	100 100 0	100 100 0	70 85 21	89 93 4.2	100 100 0	65 80 23	92 92 0	100 100 0	50 50 0
		F10	BL FL PC%	93 95 3	36 38 5	100 100 0	100 100 0	60 100 66	100 85 -15	94 88 -7	100 100 0	75 87 16	88 92 5	100 100 0	50 50 0
Normal	rhGH and EX	F11	BL FL PC%	92 91 -0.4	34 34 0	100 100 0	100 100 0	100 100 0	70 65 -7	92 86 -6	100 100 0	90 87 -4	80 84 5	100 100 0	50 50 0
	Only rhGH	F12	BL FL PC%	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -
	Neither rhGH nor EX	F13	BL FL PC%	89 87 -2	35 34 -2	100 100 0	100 100 0	60 100 67	60 45 -25	68 32 -54	100 100 0	100 80 -20	76 84 11	100 88 -13	50 50 0
		F14	BL FL PC%	65 57 -12	28 27 -4	75 85 13	100 100 0	60 60 0	15 30 100	93 93 0	100 100 0	55 65 18	56 36 -35	63 25 -60	50 50 0

BL: baseline; FL: follow up; PC%: percentage change; Total-PC: total physical component; P.F: Physical function; R.P: Role limitations due to physical health; G.H; General health; Total MC: total mental component; R.E: Role limitations due to emotional problems; E/F: energy/fatigue; E.W.B: Emotional wellbeing; S.F: social functioning

8.6.3.9 Association between change in growth, musculoskeletal parameters, metabolic, and quality of life in the first time assessment group

There was a significant positive correlation between percentage change in height and changes in TB parameters (BMC, BMD, BA, LM, FM) in all groups, but not in LS site parameters. However, the change in height showed no correlation with the changes in bone structure and geometry (pQCT) (all p>0.1).

There was a positive correlation between percentage change in LM and total density (vBMD) only in GHD who received rhGH alone or in combination with EX (r=0.8, p=0.01), Figure 8-15.

No correlations were found between percentage change in height, percentage change in bone density parameters at both sites and the change in parameters of bone turnover among both groups. Also, changes in bone biomarkers do not correlate with changes in volumetric bone density and geometric structures.

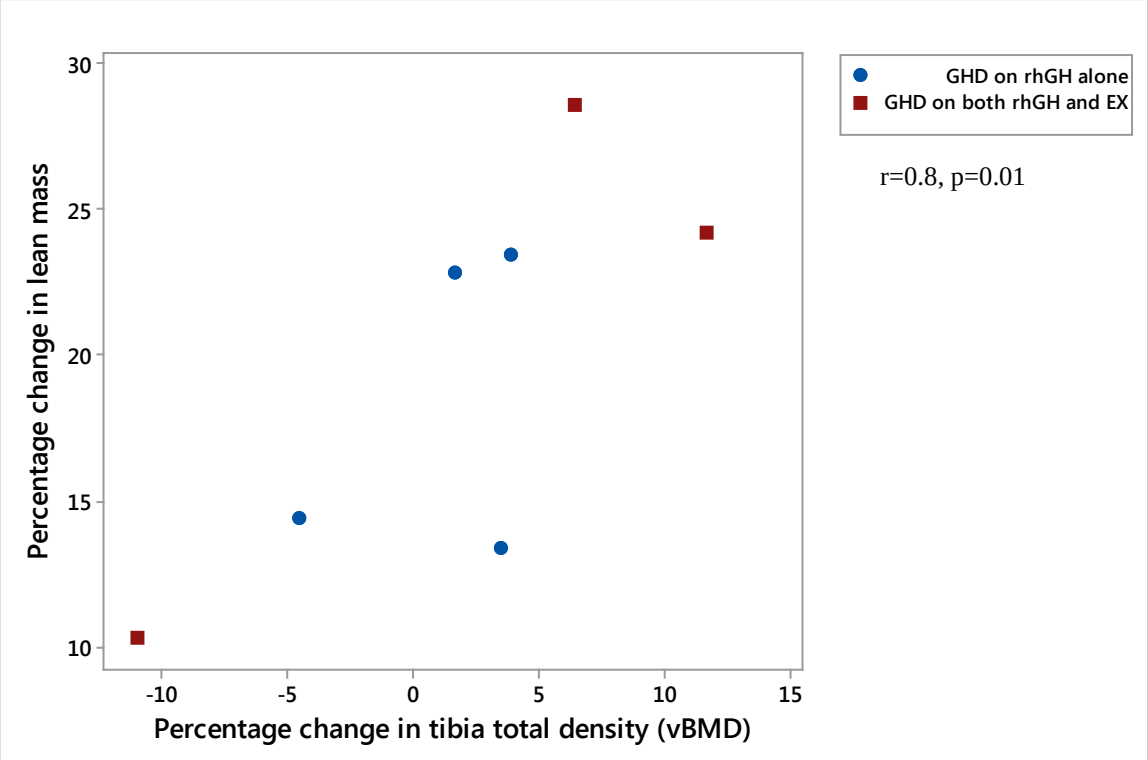

Figure 8-15 Scatterplot of the correlation between percentages changes in lean mass and tibia total density in those with GHD who received rhGH alone or in combination with exercise

8.6.4 Retesting group

8.6.4.1 The anthropometric characteristics from baseline to follow up

As was illustrated in Figure 8- 3, five of the nineteen who had completed the study were adolescents with CO-GHD at time of retesting. All five patients (two-IGHD: three- MPHD) received rhGH during childhood with median duration of 5.0 yrs (2.9, 7.8) and ceased rhGH treatment at final height to re-test their GH status at age 16.6yrs (15.2, 16.9). The median duration between off rhGH and retesting is 0.6 yrs (0.2, 0.7). All patients underwent ITT; of those, 4/5 were reconfirmed to have persistent GHD (GH peak <5mg/l), and three of them recommenced adult rhGH (adult dose: 0.2-0.5mg/d). According to randomisation, 3/5 were also allocated to exercise and 2/5 acted as controls (Figure 8-16). Clinical and demographic characteristics of these patients are shown in Table 8-10.

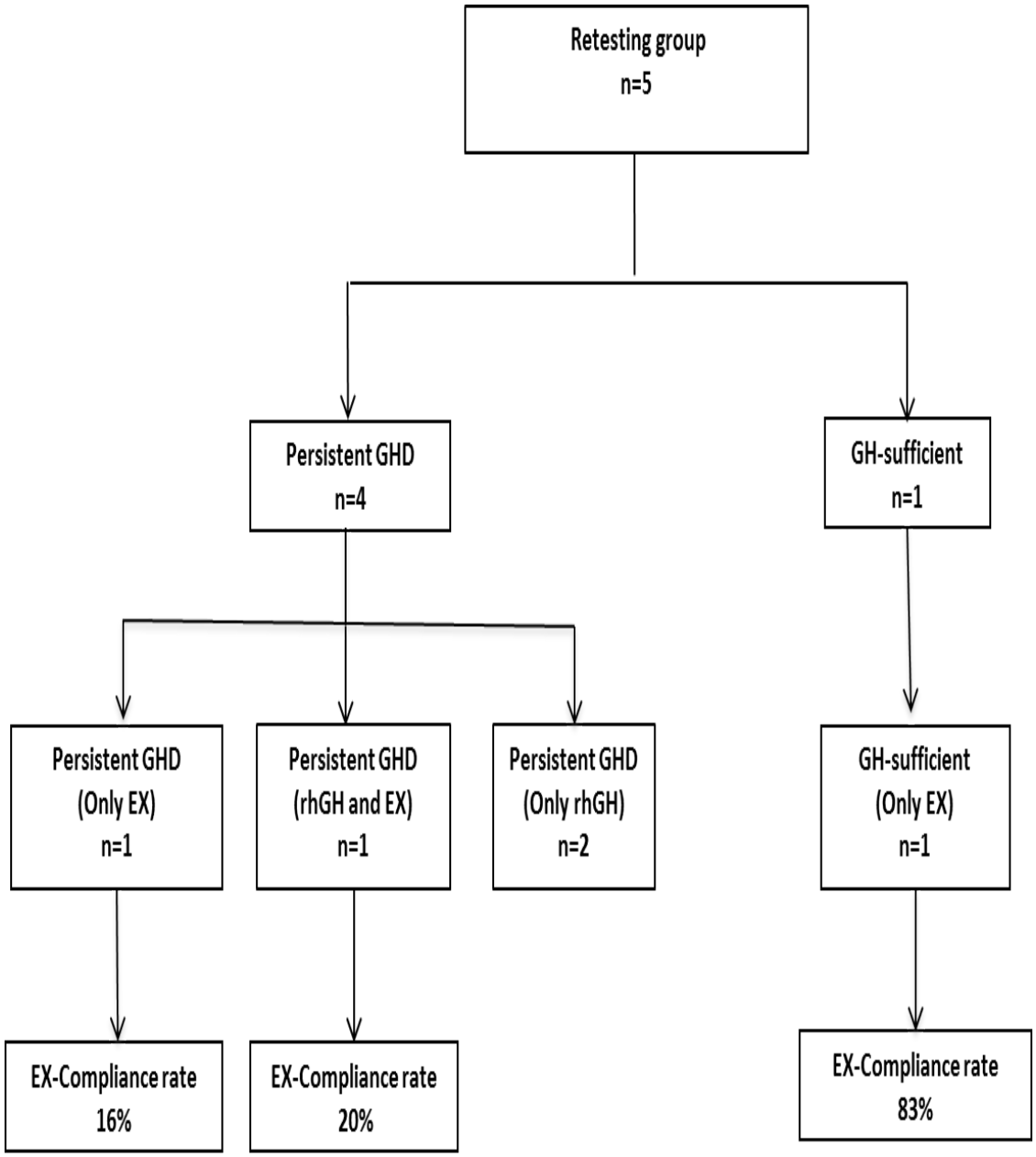

Figure 8-16 Flow chart of the retesting group.
rhGH: recombinant human growth hormone; EX: exercise

Table 8-10 Clinical and demographic characteristics of the retesting group

	Age at baseline	GHD-type (aetiology)	Duration of rhGH (yrs)	Period off rhGH	GH peak on retesting (µg/l)	Re-start-rhGH	Randomisation EX (%)	Follow up period (yr)	Duration Intervention EX/rhGH (yr)	Habitual P.A hrs/W	Co- morbidities /Medication
*R1	16.6	IGHD (Idiopathic)	5.0	0.6	3.8	-	Y(16)	1.0	1.0	5 walking machine	Celiac disease/on Vitamin D supplementation
R2	16.5	MPHD (Oncology)	2.9	0.7	3.2	Y	Y(20)	1.5	0.5	-	Thyroxine- tegretol-clobazam
R3	15.2	MPHD (Oncology)	7.8	0.2	0.8	Y	C	1.4	0.7	-	Desmopressin
R4	16.9	MPHD (Oncology)	6.3	0.7	0.2	Y	C	1.1	0.6	-	Thyroxine-testosterone
R5	16.6	IGHD (Ectopic posterior pituitary)	4.3	0.6	10.2	-	Y(83)	0.5	0.5	-	-
Median (range)	16.6 (15.2, 16.9)	-	5.0 (2.9, 7.8)	0.6 (0.2, 0.7)	-	-	26% (16, 83)	1.1 (0.5, 1.5)	0.6 (0.5, 1.0)	-	-

* R stands for retesting group

8.6.4.2 Densitometry changes

All the subjects of the retesting group had steady bone density parameters with no obvious changes from baseline to follow up and all had a value of TB/LS BMD between +2 and −2SD of normal mean, none was below -2 SD, Figure 8-17.

From Figure 8-18, (patient ID: R3), who had persistent GHD and was on only rhGH, showed the highest gain in TB-BMD/BMC (PC%: 3.8 %, 5.2%, respectively). On the other hand, (patient ID: R1), who had persistent GHD with only EX and poor compliance rate (16%), showed the highest gain in LS-BMC (PC%; 12%).

The individual data of TB and LS parameters are shown in Table 8-11

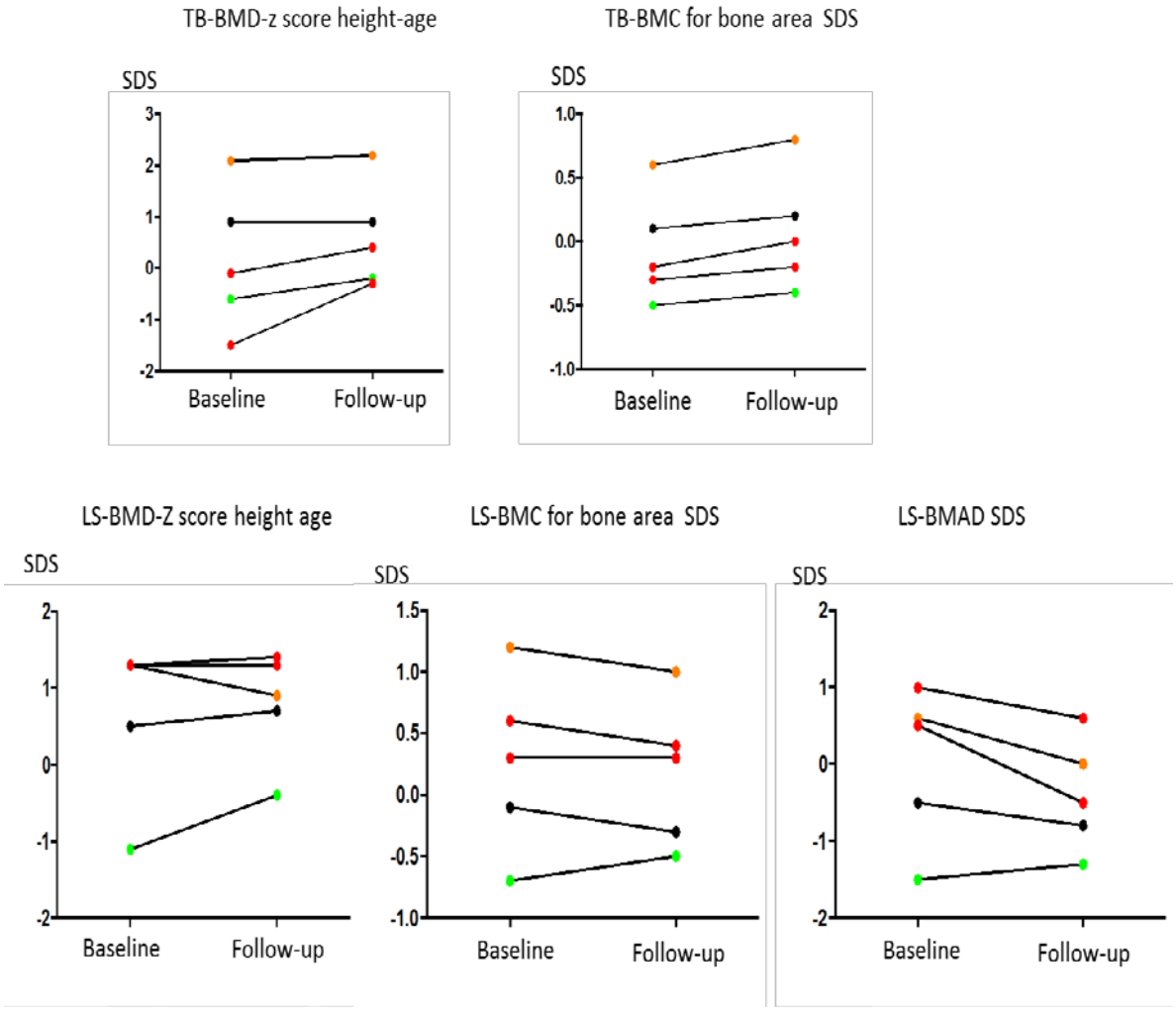

Figure 8-17 Individual data of total body and lumber spine BMC/BMD SDS from baseline to follow up in the retesting group.

Orange dot = R1: Persistent GHD (Only EX)

Green dot = R2: Persistent GHD (rhGH and EX)

Red dots =R3, R4: Persistent GHD (Only rhGH)

Black dot = R5: GH-sufficient (Only EX)

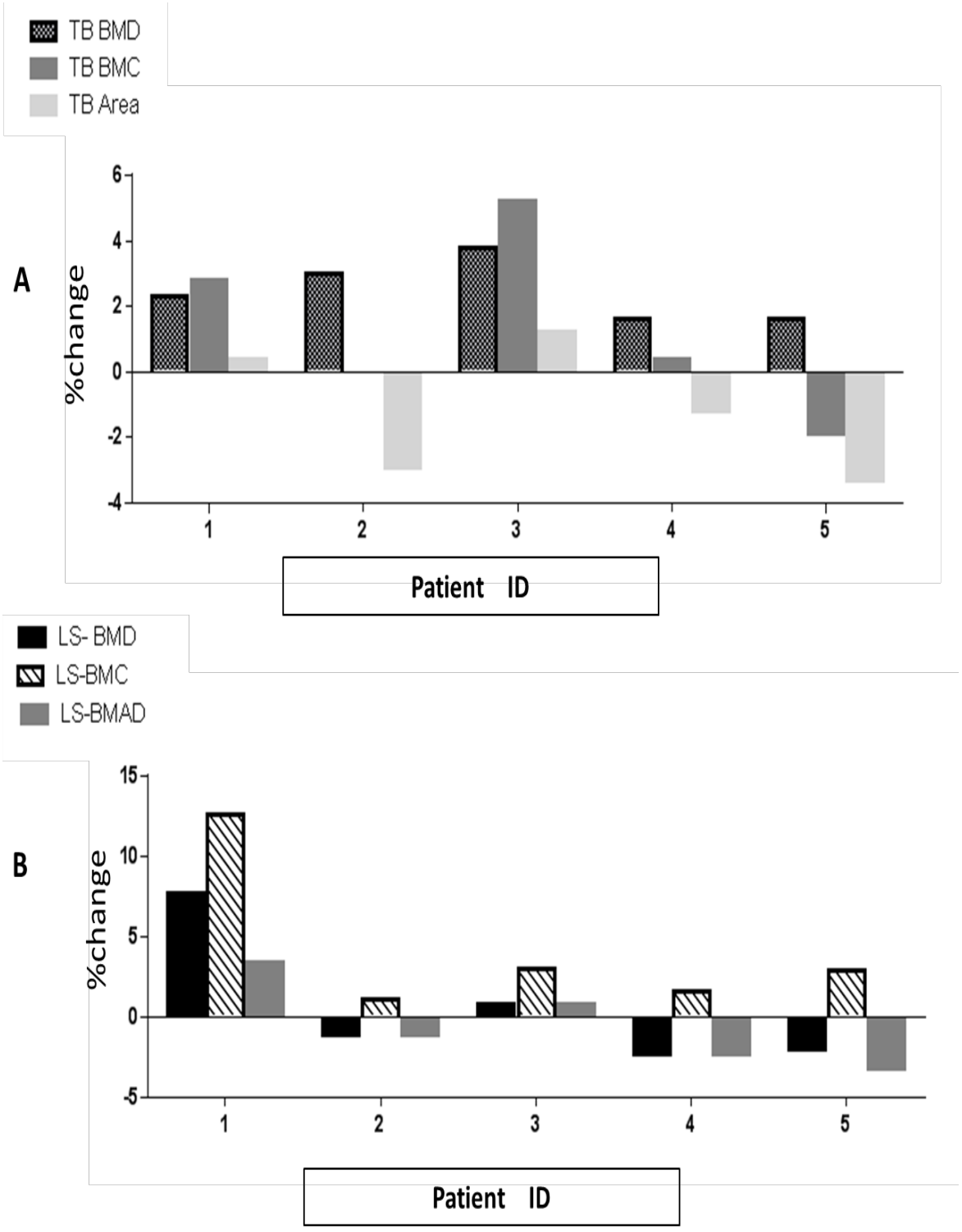

Figure 8-18 Percentage changes in TB-BMD, BMC, and bone area (A), LS-BMD, BMC, and BMAD from baseline to follow up of the retesting group.

1: Persistent GHD (Only EX); 2: Persistent GHD (rhGH and EX); 3: Persistent GHD (Only rhGH; 4: Persistent GHD (Only rhGH); 5: GH-sufficient (Only EX).

Table 8-11 Total body and lumber spine DXA bone density parameters at baseline, follow up and percentage changes of the retesting group

	Retesting group n=5						
		Time	**R1**	**R2**	**R3**	**R4**	**R5**
TB	TB-BMD (g/cm^2)	BL FL PC%	0.98 1.007 2.3	1.23 1.267 3.0	1.079 1.12 3.8	1.012 1.028 1.6	1.08 1.097 1.6
	TB-BMC (g)	BL FL PC%	1992.3 2047.5 2.8	3051.0 3050.4 0.0	2552.1 2685 5.2	2004.9 2012.9 0.4	2162.8 2121.5 -1.9
	TB-BA (cm^2)	BL FL PC%	2025 2033 0.4	2481 2408 -2.9	2366 2395 1.2	1982 1958 -1.2	2001 1934 -3.3
	TB-BMC for BA SDS	BL FL PC%	-0.5 -0.4 -20	0.6 0.8 33	0.2 0.0 -100	-0.3 -0.2 -33	0.1 0.2 100
LS	LS-BMD (g/cm^2)	BL FL PC%	0.905 0.975 7.7	1.209 1.196 -1.1	1.184 1.193 0.8	1.149 1.123 -2.3	1.114 1.092 -2.0
	LS-BMC (g)	BL FL PC%	32.1 36.13 12.2	43.32 43.79 1.1	57.31 59.04 3.0	44.22 44.94 1.6	44.67 45.96 2.9
	LS-BA (cm^2)	BL FL PC%	35.45 37.06 4.5	35.82 36.62 2.2	48.4 34.91 -27.9	38.48 40.01 4.0	40.11 42.07 4.9
	LS-BMC for BA SDS	BL FL PC%	-0.7 -0.5 -28	1.2 1.0 -16	0.3 0.3 0	0.6 0.0 -100	-0.1 -0.3 200
	LS-BMAD (g/cm^3)	BL FL PC%	0.325 0.336 3.4	0.354 0.350 -1.1	0.351 0.353 0.8	0.348 0.341 -2.3	0.369 0.357 -3.2
	LS-BMAD SDS	BL FL PC%	-1.5 -1.3 -12.8	0.6 0.6 -10.9	1.0 0.6 -40.0	-0.5 -0.5 17.5	-0.5 -0.8 35.2

R1: Persistent GHD (Only EX)

R2: Persistent GHD (rhGH and EX)

R3: Persistent GHD (Only rhGH)

R4: Persistent GHD (Only rhGH)

R5: GH-sufficient (Only EX)

8.6.4.3 Changes in body composition measured by DXA

LM did not obviously change from baseline to follow up in the majority of the group. The patient ID (R4) who had persistent GHD and restarted rhGH showed the highest gain in LM from baseline to follow up compared to the others (PC% =12.7%), when the lowest percentage change in LM (PC%= 0.7%) was recorded in (patient ID: R1) with persistent GHD, but only EX (compliance 16%). FM was not changed in the majority of the subjects with only (patient ID: R5, GH sufficient on only EX) having the highest loss in FM (PC%= -11.4%), from 24.7 kg at baseline to 21.8 kg on follow up.

The changes in A/G ratios were various among the group as seen in Figures 8-19, 8-20.

The individual data of body composition parameters at baseline and follow up are shown in Table 8-12.

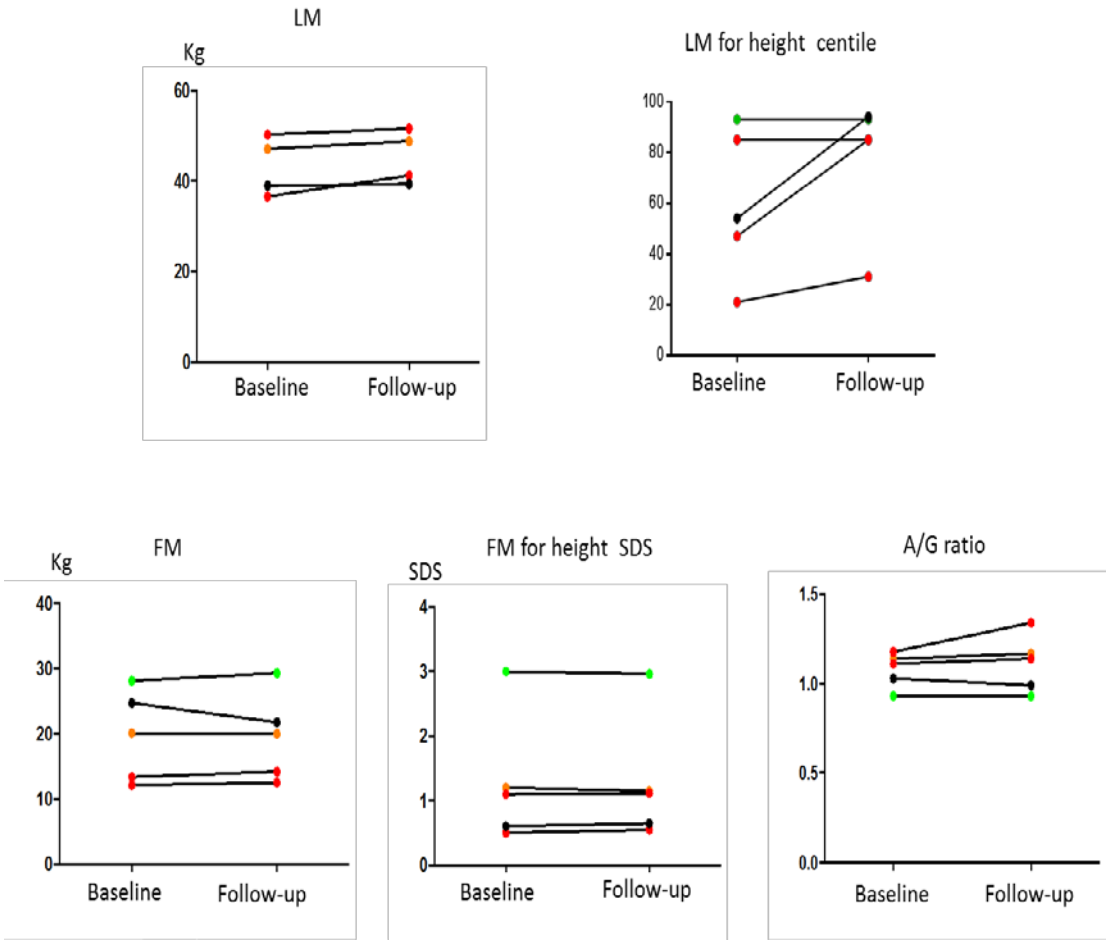

Figure 8-19. Individual data of body composition from baseline to follow up in the retesting group.

Orange dot = R1: Persistent GHD (Only EX)

Green dot = R2: Persistent GHD (rhGH and EX)

Red dots =R3, R4: Persistent GHD (Only rhGH)

Black dot = R5: GH-sufficient (Only EX)

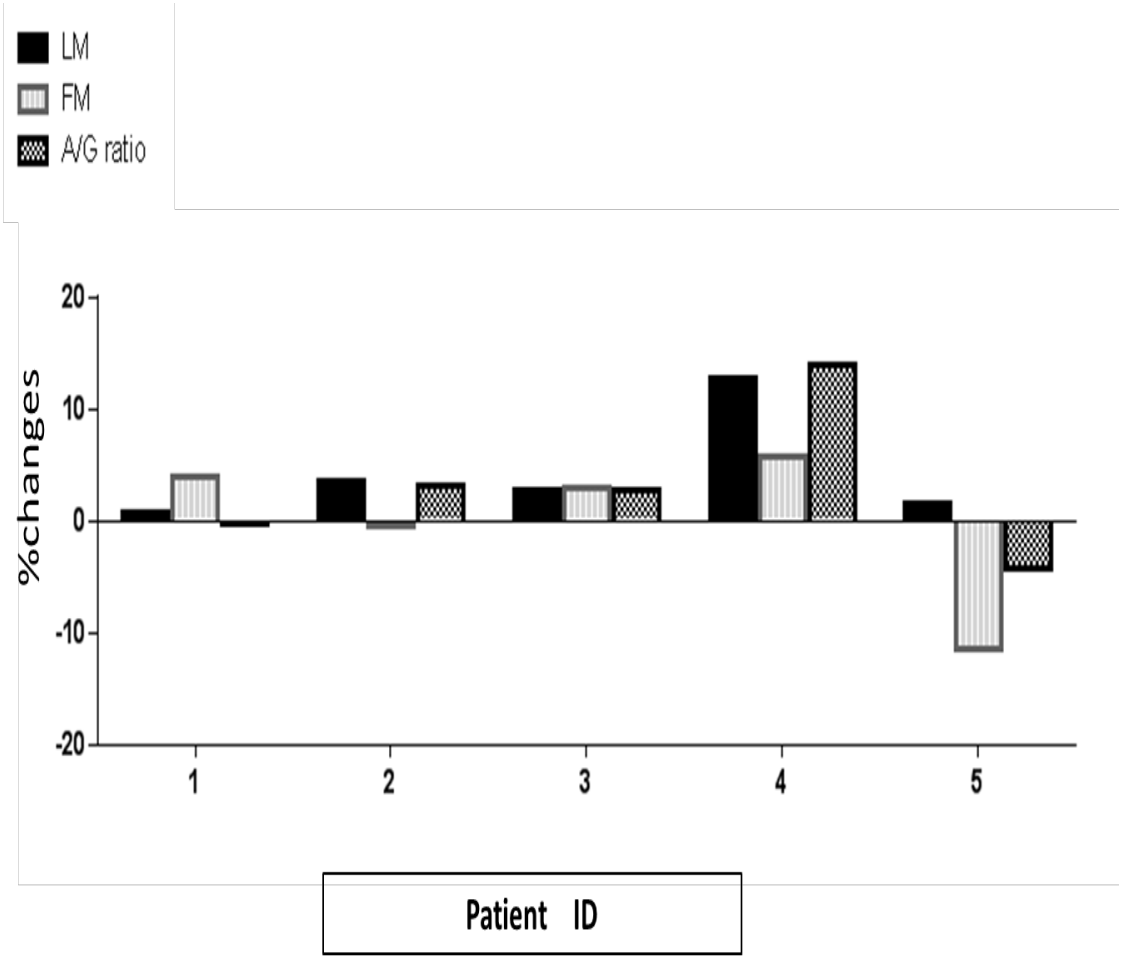

Figure 8-20 Percentage changes in LM, FM and A/G ratio from baseline to follow up of the retesting group.

1: Persistent GHD (Only EX); 2: Persistent GHD (rhGH and EX); 3: Persistent GHD (Only rhGH; 4: Persistent GHD (Only rhGH); 5: GH-sufficient (Only EX).

Table 8-12 Individual data of DXA-body composition parameters at baseline, follow up and percentage changes of the retesting group.

	Retesting n=5						
		Time	**R1**	**R2**	**R3**	**R4**	**R5**
LM	LM (kg)	BL FL PC%	39.0 39.3 0.7	47..1 48.8 3.5	50.2 51.6 2.8	36.5 41.2 12.7	38.9 39.4 1.5
	LM for height centile	BL FL PC%	93.0 93.0 0.0	85.0 85.0 0.0	21.0 31.0 47.6	47 85 80.9	54 94 74.1
FM	FM (kg)	BL FL PC%	28.1 29.3 4.0	20.1 20.0 -0.4	12.1 12.5 3.0	13.4 14.2 5.8	24.7 21.8 -11.4
	FM for height SDS	BL FL PC%	2.8 3.0 -	1.2 1.2 -	0.5 0.5 -	1.0 1.1 -	1.1 0.6 -
	A/G ratio	BL FL PC%	0.93 0.93 -0.3	1.14 1.17 3.3	1.11 1.14 2.8	1.18 1.34 13.9	1.03 0.99 -4.1

BL: baseline; FL: follow up; PC%: percentage change; LM: lean mass; FM: fat mass; A/G: Android/Gynoid fat ratio

8.6.4.4 Tibia bone and muscle parameters

The patient ID (R1) has no pQCT at baseline so she was excluded. Of the remaining four patients, there was variation but no remarkable changes concerning bone structure and geometry. Cortical thickness, periosteal circumference and endoeosteal circumference were somewhat various in the group (Table 8-13, Figure 8-20). Similarly, there were no apparent changes in the parameters of tibia bone density

Muscle CSA increased in patient IDs: R2, R4, and R5, with patient ID: R4 having the highest muscle CSA percentage change, gaining 21% from his baseline value. Conversely, patient ID: R3 lost -2.6 % of his muscle CSA from baseline to follow up, but had the highest gaining in fat CSA from baseline to follow up (1269.25 mm2 to 1615.00 mm2, PC% = 27.2%). Fat CSA was only decreased in patient ID: R2 from 2168.75 mm2 to 1815.00 mm2, PC% = -16.3%.

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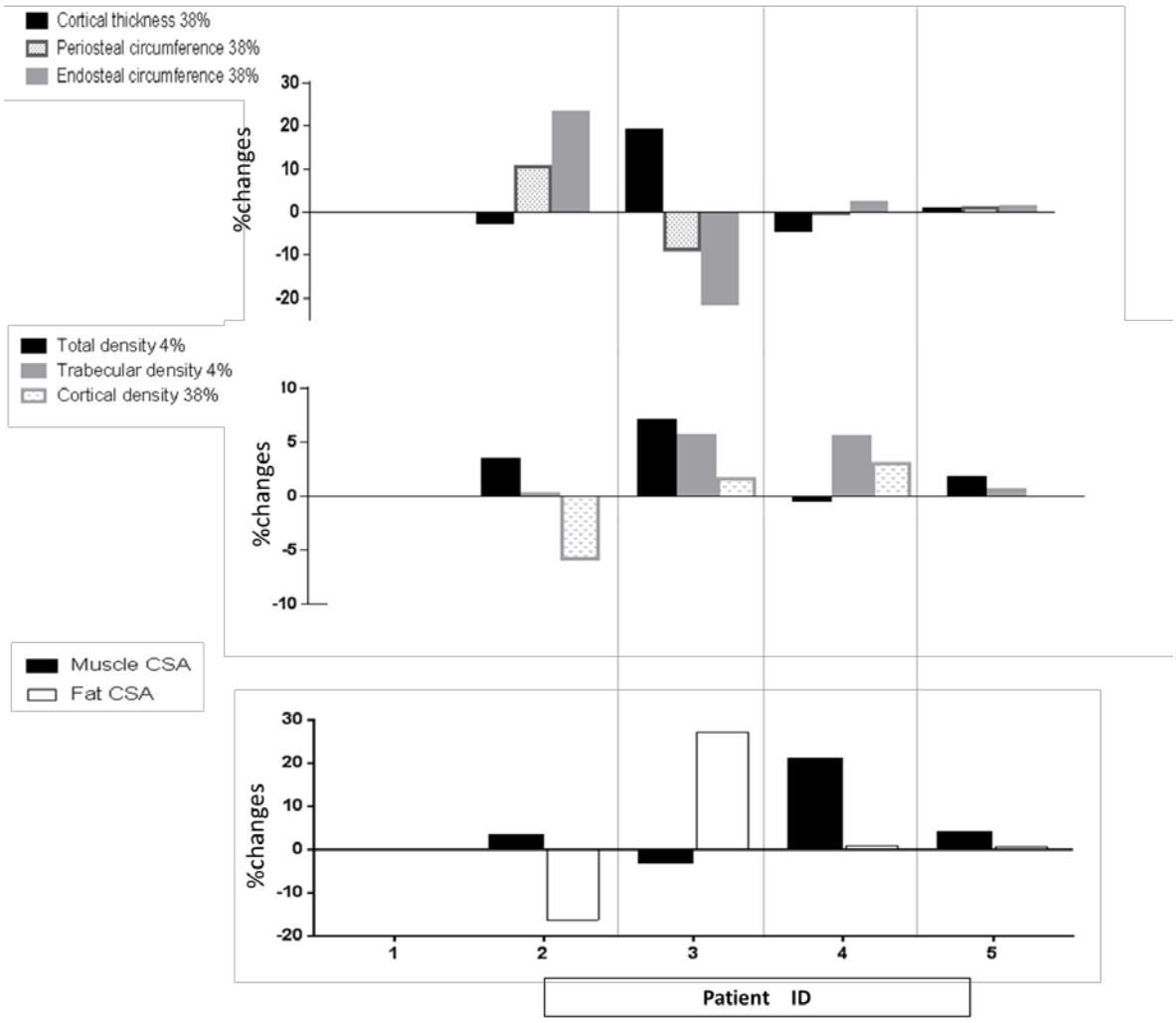


Figure 8-21 Percentage changes in tibia pQCT parameters from baseline to follow up of the retesting group.
1: Persistent GHD (Only EX); 2: Persistent GHD (rhGH and EX); 3: Persistent GHD (Only rhGH; 4: Persistent GHD (Only rhGH); 5: GH-sufficient (Only EX)

Table 8-13 Tibia pQCT parameters at baseline, follow up and percentage changes of the retesting group

Retesting n=5						
	Time	**R1**	**R2**	**R3**	**R4**	**R5**
CTh -38% (mm)	BL FL PC%	- 3.7 -	5.9 5.8 -2.5	3.9 4.6 18.9	3.9 3.8 -4.1	4.3 4.3 0.6
CTh -38% Height-z score	BL FL PC%	- -0.8 -	1.0 0.9 -	-0.7 -0.2 -	-0.1 -0.2 -	1.3 0.5 -
PC -38% (mm)	BL FL PC%	- 65.7 -	74.5 82.2 10.4	77.5 70.8 -8.6	66.7 66.6 -0.2	64.2 64.8 1.0
PC -38% Height-z score	BL FL PC%	- -0.5 -	0.2 1.6 -	-0.6 -2.1 -	-0.8 -0.9 -	0.3 -0.7 -
EC -38% (mm)	BL FL PC%	- - -	37.6 46.2 23.0	53.2 41.7 -21.2	41.7 42.6 2.1	37.3 37.8 1.3
PC -38% Height-z score	BL FL PC%	- 0.2 -	-1.8 0.0 -	1.0 -1.3 -	-0.5 -0.3 -	-0.6 -0.9 -
TvBMD 4 % (mg/cm^3)	BL FL PC%	- 264.6 -	351.1 362.9 3.4	227.8 243.9 7.0	294.9 293.6 -0.4	308.8 314.1 1.7
TrvBMD 4% (mg/cm^3)	BL FL PC%	- 210.7 -	268.5 269.0 0.2	193.6 204.5 5.6	230.8 243.5 5.5	252.1 253.6 0.6
TrvBMD 4% Age-z score	BL FL PC%	- -0.4 -	0.7 0.4 -	-1.2 -1.2 -	-0.4 -0.4 -	0.7 0.8 -
CvBMD 38% (mg/cm^3)	BL FL PC%	- 1154.2 -	1154.5 1087.6 -5.8	1158.0 1176.0 1.6	1123.68 1156.95 3.0	1171.0 1171.3 0.0
CvBMD 38% Age-z score	BL FL PC%	- 0.3 -	1.7 1.7 -	2.2 2.3 -	0.5 1.0 -	0.6 0.7 -
SSI 38%	BL FL PC%	- 1190.4 -	1558.0 1524.5 -2.2	1263.9 1549.9 22.6	1145.4 1133.1 1.1	1193.8 1202.4 0.7
Cortical CSA 66% (mm2)	BL FL PC%	- 288.0 -	445.2 419.0 -5.0	303.2 316.5 4.4	267.5 275.5 3.0	304.8 309.0 1.4
Muscle CSA 66% (mm^2)	BL FL PC%	- 5107.0 -	8176.2 8418.0 3.0	6117.0 5939.8 -2.6	5220.8 6310.0 20.9	5247.2 5448.8 3.8
Fat CSA 66% (mm^2)	BL FL PC%	- 3543.0 -	2168.8 1815.0 -16.3	1269.2 1615.0 27.2	2755.5 2781.0 0.9	2817.2 2836.8 0.7

BL: baseline; FL: follow up; PC%: percentage change; CTh: Cortical thickness; PC: Periosteal circumference; EC: Endosteal circumference; TvBMD: Total density; TrvBMD: Trabecular density; CvBMD: Cortical density; SSI: Strength strain index;

8.6.4.5 Changes in bone profiles and metabolism

Ca, Po4 and Mg levels were within the reference range and not obviously changed from baseline to follow up in the majority of subjects. PTH levels were slightly decreased with increased vit-D in patient IDs: R1, R2 and R3 during the study period. Bone metabolic biomarkers were various among the group, Table 8-14.

8.6.4.6 Changes in metabolic profiles

From Table 8-15, a rise in total cholesterol and LDL was observed in (patient IDs R2, R3, R4), who were on rhGH during the study period, particularly (patient ID: R3) showed higher level of total cholesterol on follow up compared to baseline value.
FFA levels decreased from baseline values in all patients except (patient ID: R5) who showed a 22.9% higher FFA levels from baseline to follow up.

Similarly, leptin levels rose from baseline to follow up in those who were on rhGH, when (patient ID: R1) who had GHD but did not receive rhGH yet showed a lower leptin level nearly half of the baseline value. Adiponectin levels were steady in (patient IDs: R1, R2, R3), but decreased in (patient IDs: R4 and R5), unlike resistin which was mostly decreased in (patient ID: R1) but increased in patient ID: R5 during the study period.

With regard to glucose homeostasis parameters, (patient ID: R1) showed the highest decrease in both fasting insulin and HOMA IR from baseline to follow up compared to the rest of the group. Of the remaining, fasting insulin and HOMA-IR reduced in both (patient IDs: R2 and R5), but increased in (patient ID: R4) the values of baseline to follow up.

Table 8-14 Bone profiles and mineralisation at baseline, follow up and percentage changes of the retesting group

		Retesting (n=5)					
		Time	**R1**	**R2**	**R3**	**R4**	**R5**
Bone profiles	Ca mmol/l	BL FL PC%	2.3 2.3 0.9	2.5 2.5 -0.8	2.4 2.4 -1.2	2.5 2.4 -4.8	2.3 2.3 0.0
	Po4 mmol/l	BL FL PC%	0.9 1.2 34.8	0.85 1.02 20.0	0.98 1.18 20.4	1.0 0.9 -15.9	0.9 0.9 1.0
	Mg mmol/l	BL FL PC%	0.8 0.9 7.2	0.9 0.9 -1.1	0.9 0.8 -3.3	0.8 0.8 -4.7	0.7 0.7 -5.5
	PTH pmol/l	BL FL PC%	6.4 5.1 -19.5	4.3 3.0 -30.2	3.4 3.7 8.5	6.7 3.9 -42.1	9.2 7.6 -17.4
	25OH vit-D (nmol/l)	BL FL PC%	55.0 59.0 8.5	37.0 87.0 135.1	32.0 36.0 13.4	33.0 30.7 -7.0	23.0 22.0 -4.3
Bone biomarkers	BAP µg/l	BL FL PC%	11.7 13.1 11.8	33.6 23.1 -31.1	23.7 44.8 88.4	81.1 56.7 -26.4	15.6 24.4 55.7
	OC ng/ml	BL FL PC%	25.4 18.9 -25.5	33.2 26.2 -21.1	34.1 41.7 22.2	35.0 21.3 -39.2	14.9 15.3 2.7
	CTX ng/ml	BL FL PC%	1.5 0.3 -74.7	1.0 3.5 247.3	0.6 1.2 85.1	1.6 1.4 -13.1	1.9 1.7 -10.0

BL: baseline; FL: follow up; PC%: percentage change; Ca: calcium; PO4: phosphate; Mg: magnisum; PTH: parathyroid hormone; 25 (OH) Vit-D: 25 hydroxy vitamin D; BAP: bone-specific alkaline phosphatase; OC: osteocalcine; CTX: cross linked C-telopeptide of type I collagen

Table 8-15 Metabolic profiles, adipokines and glucose homeostasis parameters at baseline, follow up and percentage changes of the retesting group

		Retesting (n=5)					
		Time	**R1**	**R2**	**R3**	**R4**	**R5**
Lipids profiles	Total cholesterol mmol/L	BL FL PC%	3.4 3.3 -5.2	4.7 5.4 14.6	3.3 4.54 37.6	3.9 4.8 22.1	4.1 3.6 -12.2
	HDL mmol/L	BL FL PC%	1.0 0.9 -15.9	1.2 0.9 -25.6	0.87 1.0 14.9	1.21 1.1 -9.1	1.3 1.3 0.0
	LDL mmol/L	BL FL PC%	2.0 2.1 5.0	2.9 3.6 23.3	1.9 3.11 57.9	2.1 2.8 30.2	2.5 2 -20.0
	TG mmol/L	BL FL PC%	1.1 0.6 -45.5	1.2 1.9 49.6	1.0 0.9 -6.9	1.2 - -	0.7 0.6 -14.3
	FFA mmol/L	BL FL PC%	0.3 0.2 -21.2	0.3 0.2 -16.7	0.4 0.3 -18.6	0.44 - -	0.3 0.4 22.9
Adipokines	Leptin ng/ml	BL FL PC%	37.4 15.4 -58.8	11.8 13.2 11.0	2.0 3.2 55.9	8.2 11.4 39.1	24.6 24.6 -0.2
	Adiponectin ng/l	BL FL PC%	8.0 7.8 -2.6	3.3 3.2 -0.9	2.8 2.8 -1.0	1.7 1.0 -40.4	6.2 3.2 -47.0
	Resistin ng/ml	BL FL PC%	22.2 8.2 -63.2	3.8 2.5 -32.9	3.9 3.9 0.2	2.9 3.0 5.8	6.7 8.3 22.9
Glucose metabolism	F-Glucose mmol/L	BL FL PC%	4.2 3.8 -9.5	4.5 4.8 6.7	4.2 4.8 14.3	5.6 4.6 -17.9	4.4 5.1 15.9
	F-Insulin uIU/ml	BL FL PC%	45.8 20.2 -55.9	32.8 22.2 -32.2	10.2 11.1 8.8	- 44.9 -	33.1 21.9 -33.8
	HOMA-IR	BL FL PC%	8.5 3.4 -60.1	6.6 4.7 -27.6	1.9 2.4 24.4	- 9.2 -	6.5 5.0 -23.3

BL: baseline; FL: follow up; PC%: percentage change; LDL: low density lipoprotein cholesterol; HDL: high density lipoprotein cholesterol; TG; triglyceride; FFA; free fatty acid; F- glucose: fasting glucose; F- insulin: fasting insulin; HOMA-IR:homeostasis model assessment insulin resistance index

8.6.4.7 Changes in quality of life measures (AGHDA)

Looking for individual AGHDA scores in Figure 8-22, Table 8-16, all patients except one (patient's ID: R2) had poorer scores at follow-up compared to their baseline scores regardless of whether they were on rhGH or not. The patient's ID: R2 (persistent GHD on combined rhGH+EX) was the only patient who achieved improvement in his total QoL scores from baseline to follow up by - 8 points. Unlike, patient's ID: R1 (persistent GHD with only EX) and patient's ID: R5 (GH sufficient and only EX) who had reported poorer QOL scores on follow up (+6 points, +5 points, respectively). From aspects of AGHDA, the scales of memory and tiredness are mostly affected in our subjects. The patient's ID R1 and R5, reported reduced memory and concentration (+3 points, +4 points: respectively) from their baseline levels, when patient's ID R3 (persistent GHD on only rhGH) and patient's ID: R5 (GH sufficient and only EX) showed poorer scores on the scale of tiredness from baseline (+3points each).

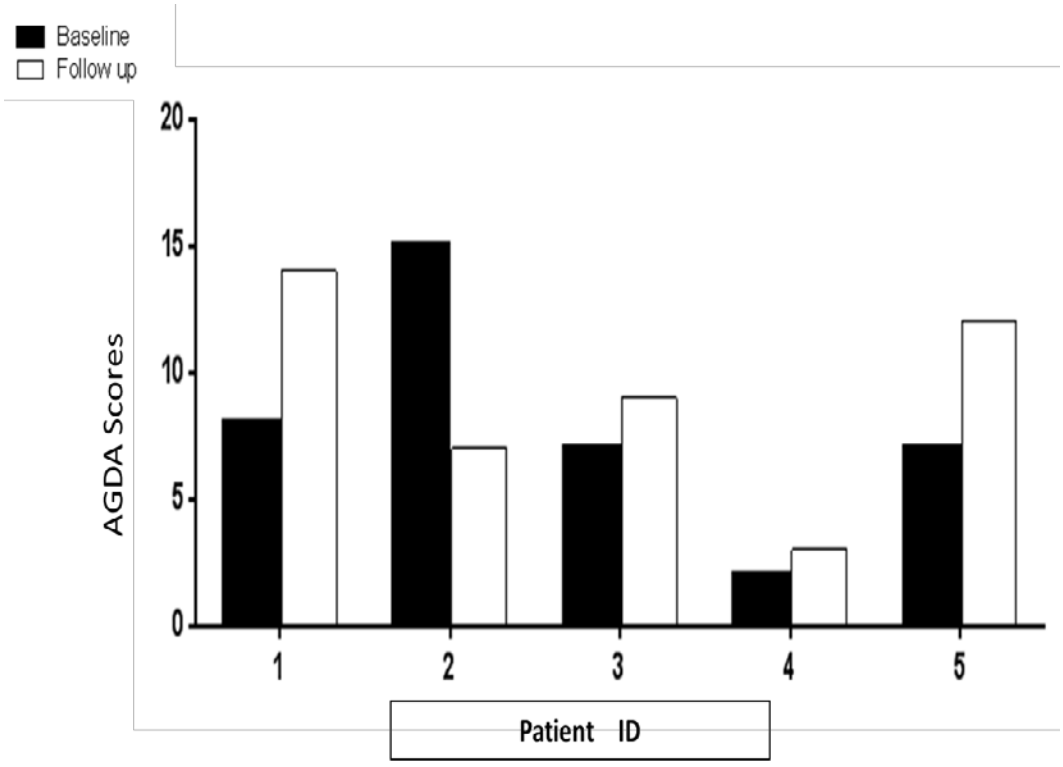


Figure 8-22 Individual QoL-AGHDA scores at baseline and follow up in the retesting group.
(Higher scores reflect poorer QoL)

Table 8-16 AGHDA scores of quality of life assessment at baseline, follow up and absolute changes of the retesting group.
(Higher scores reflect poorer QoL)

Subscale	Retesting (n=5)					
	Time	**R1**	**R2**	**R3**	**R4**	**R5**
Total scores (25)	BL FL AC	8 14 +6	15 7 -8	7 9 +2	2 3 +1	7 12 +5
Memory and concentration(6)	BL FL AC	2 5 +3	5 3 -2	1 0 -1	0 0 0	0 4 +4
Tiredness(7)	BL FL AC	1 1 0	7 4 -3	1 4 +3	0 0 0	3 6 +3
Tenseness(3)	BL FL AC	1 2 +1	0 0 0	2 2 0	0 0 0	2 1 -1
Social isolation(5)	BL FL AC	3 3 0	1 0 -1	3 3 0	2 2 0	2 0 -2
Self-confidence(4)	BL FL AC	1 3 +2	2 0 -2	0 0 0	0 1 +1	0 1 +1

BL: baseline; FL: follow up; AC: absolute change

8.6.4.8 Association between change in musculoskeletal parameters, metabolic, and quality of life in the retesting group

In the retesting group, no significant correlations were found between changes in bone metabolism parameters and changes in bone structure. There were also no statistically significant correlations between percentage changes of bone, body composition and metabolism parameters observed either with being treated with rhGH or the exercise regimen.

8.7 Discussion

There is increasing evidence that WBE, as simple as a jumping regimen, represents a very important mechanical positive influence on bone mass and bone size in growing children and adolescents (281,286). Recently, WBE has become one of the strategies for prevention and treatment of osteoporosis in several conditions: menopausal; diabetes, cancer and other metabolic diseases (507-510). However, the interaction between WBE, GHD and rhGH has not been studied before, with the growing concern of the cost effectiveness of rhGH on health aspects of adolescents with CO-GHD during transition of adolescents with CO-GHD after attaining final height.
Numerous previous studies of rhGH therapy in adolescents with persistent GHD beyond final height reported a net benefit change in LS-BMD with rhGH about 3 to 6 % after one (227) or two years (230), which is similar in magnitude to the gain in LS- BMD (4.5%) observed in the jumping exercise at 7-months (286). This is supporting the potential clinical relevance of suggesting WBE as an alternative or complementary strategy that may enhance bone health. Therefore, in this pilot study we have investigated the short term effects of either/or combined rhGH with WBE exercise on children and adolescents with CO-GHD.
Since we hypothesized WBE would enhance bone health parameters, we were not able to show definite efficacy of WBE in bone or any other health aspect parameter of GHD for several reasons. Disappointingly poor compliance to the exercise program seems to be important for the lack of impact on the primary end points. In addition to poor compliance rate, the small sample size made it difficult to establish any significant differences between groups. Additionally, the short duration of exercise intervention particular combined with rhGH could be an issue due to short term bone changes of rhGH (remodelling) (44). The available data suggest, however, that the WBE could make a significant contribution towards marked changes in body composition (increase LM, decrease FM) of patients with GHD but in combination with rhGH.
Additionally in this study, our ambition was not to only test WBE, rather, this study was designed to examine the feasibility of using WBE in a clinical setting and to generate empirical evidence, on which hypotheses can be based in future, large-scale studies. Our study revealed that the concept of exercise intervention as a replacement or complement intervention was not applicable at home; particular as it was more challenging for the parents, who needed to have a an active role with this intervention. To translate this program to a real-world setting, we would suggest an exercise intervention for these children either within a hospital setting or an alternative optimal option would be within school-based interventions (511).

On the effect of rhGH in our studied groups, as expected, in the rhGH treated first time assessment group, TB-BMC gain during rhGH was significantly lower for the gain to bone area and LM. Numerous well-controlled studies demonstrate homogenous results of initial reduction of bone density after 6-12 months of starting therapy with rhGH of children with GHD; when therapy continued, BMD increase appeared after 18 months and was sustained at 24 months (235,413). It

was suggested that reduction in bone density results from increase in bone modelling and remodelling together with the catch-up growth during the first year of rhGH therapy in GHD children (235), and rhGH causes a maximal effect on bone resorption after 3 months and on bone formation after 6 months (44). In another setting, it was reported that the growth in bone size results in relatively under mineralized bone and increased fracture risk in the pubertal years (250,512). In line with the lack of significant changes in overall rate of bone turnover, no changes were observed in TB-LS bone BMD/BMC in our adolescents group.

In terms of bone morphology and structure in response to rhGH, there is evidence that GH stimulates cortical bone apposition leading to an increase in bone mass through increasing cortical thickness and density; with limited or no influence on GH on trabecular bone (163). In contrast to the literature (235), and contrary to our initial hypothesis, we found a normal cortical density before the start of therapy, which decreased on treatment without obvious changes in trabecular density.

Our data results also confirm that rhGH significantly changes body composition with a significant gain in LM and decreased FM in those treated with rhGH compared to those who were not treated among the first time assessment group (233,513). In contrast, the discontinuation and recommencement effects of rhGH on body composition of the retesting group were not evident in our data, which was assumed to be due to the short duration off treatment and small numbers. However, a similar observation was reported previously from larger studies over a longer period up to two years showing no differences in body composition from time of withdrawal of rhGH at final height and two years, whether or not rhGH treated (232).

Besides its beneficial effects on bone and body compositions, rhGH replacement is also suggested to alleviate at least some of the aspects of metabolic profile lipids, adipokines and glucose homeostasis. Total and HDL cholesterol were comparable between rhGH- and non rhGH- GHD of the first time assessment and retesting subjects and that did not change significantly over the period of follow up, similar to what was previously reported (514). Unlike other studies which showed significant favourable changes in lipids profiles over one year of rhGH therapy in children (261,441,450) and adolescents with CO-GHD (442).

The action of GH on adipose tissue is documented by its effects on adipokines secreted by adipocytes such as leptin, adiponectin, and resistin which are known to play an important role in glucose homeostasis as well as bone metabolism (144). However, there were insignificant reductions in the levels of leptin in the course of rhGH treatment which have been associated with decreases in FM in those with GHD treated with rhGH. These results were comparable to those previously reported in children (440,450,451,463) and adults with CO-GHD (515). Similarly, it is well documented that GH has antagonistic effects on insulin, and increased insulin resistance has been reported as a possible negative effect of GH treatment (444). Relatively few studies have investigated insulin sensitivity in children with GHD during rhGH replacement therapy, with

inconsistent findings (450,462,516). In subjects treated with rhGH, there is a slight reduction in insulin sensitivity, as measured by HOMA-IR, which was related with decreased insulin levels. However, it is well documented that rhGH induced insulin resistance is counteracted by an increase in LM and a decrease in FM (138). In our retesting cohort, those with persistent GHD were more sensitive to insulin than those with normal growth hormone secretion. From the literature, rhGH induced insulin resistance reported in GHD adults (517) was not observed in adolescents (518) .

The beneficial effects of rhGH replacement on QoL of individuals with GHD are less conclusive. Studies have showed that rhGH treated GHD showed greater self-esteem compared to short stature and normal stature children (482,496). However, our data, in keeping with other (492,519) , did not show any different in QoL measures of subjects receiving rhGH therapy. However, a slightly diminished in the aspect of emotional wellbeing was observed in some of subjects receiving hGH in our group. This finding could be explained, in part, by the emotional distress of repeated injections in those children and parents, similar to what was previously reported in children and families with type 1diabetes (520).

With regarding our retesting group, the majority of our subjects' demonstrated rather poorer QoL AGHDA scores on follow up compared to baseline. Reviewing the literature, it was previously reported that there is an inverse relationship between QoL and duration of off rhGH therapy with a longer period off rhGH associated with a poorer QoL (264); whereas, re-instituting rhGH treatment has a significant positive change in health related QoL aspects (242,264). A study reported that rhGH replacement therapy has a beneficial effect on attentional performance in adult patients with GHD when treated for at least 3 months (521). Some studies indicate less improvement in QoL of patients with CO-GHD than those with adult-onset disease (247) even after long term rhGH therapy (4-10 years) (522).

The current study had several limitations. The very small sample size was, in our opinion, too limited to explore the effects of weight bearing exercise. It does demonstrate very well the challenges of getting good compliance in a weight bearing intervention at home, and this would be a better significant finding for the significance of the first pilot study. Another limitation is that we have not measured the habitual physical activity.

8.8 Conclusion

There was insufficient evidence to recommend the use of jumping exercise in the absence of rhGH in children and adults with GHD. Our data suggest that jumping exercises may be more beneficial when combined with rhGH in these subjects. This requires further large studies to explore the interactions between rhGH and exercise training on bone health and body composition in CO-GHD. In addition, this study underlines the short term beneficial effects of rhGH on not only the growth, but also body composition and metabolism of children with CO-GHD.

CHAPTER 9

General Discussion and Future Directions

9.1 General Discussion

The primary object of this thesis was to study bone health and body composition of children and adolescents with CO-GHD at the time of initial evaluation and at retesting after withdrawal of rhGH therapy at final height. There is currently conflicting evidence in the existing literature that CO-GHD may contribute to low bone mass and increase fracture risk in adulthood (227,233,523), although the pathophysiology and mechanism of reduced bone mass is not fully explained. Indeed, the conflicting data on bone mass in CO-GHD might be affected by many factors such as age at onset, gender, height, body composition, gonadal status, the severity of GHD, and assessment methods. This thesis comprised several hypotheses which were explored in six studies with different groups of subjects with CO-GHD.

In chapter 3, we retrospectively reviewed the management of CO-GHD in Scotland from 2005 to 2013 after patients reached their final height. Our aims were to assess the incidence of, and to determine the predictors of persistent GHD in patients with CO-GHD after retesting at final height. Our data showed a substantial proportion (82%) of the retested patients with CO-GHD continue to have GHD as adults and most opt for GH therapy as adults. Our data also confirm that there are no clear auxological or clinical signs that predict the transiency or the persistence of GHD except for a history of organic disease and the presence of two or more additional PHDs, presence of hypothalamic-pituitary structural abnormalities and tumour related organic GHD (199,206,368-371). This study also raises a concern about follow-up of those who no longer have GHD, and patients with persistent GHD who opted not to resume adult rhGH therapy. Follow-up studies are needed for both of those groups of subjects. Despite the availability of clinical guidelines, there was significant variation in clinical practice of the management of CO-GHD between the four Scottish centres. The findings of this study suggest that the optimal management of adolescents with CO-GHD requires continuous follow-up during transition and effective communication between paediatric and adult services. In addition, appropriate re-evaluation during transition remains a crucial concept for continuing rhGH therapy in those with persistent CO-GHD.

Bone health and body composition of subjects with CO-GHD was explored in chapter 4. A retrospective analysis of DXA results of 21 childhood- treated adolescents with CO-GHD who have attained final height between 2005-2013 in the Royal Hospital for Children, Glasgow, compared with 21 age, gender and height matched healthy controls. After adjusting for several confounders (age, height, bone size and body composition), our results revealed that despite childhood rhGH replacement, adolescents with CO-GHD have a bone mass deficit after reaching their final height compared with controls. Our results also suggest that the beneficial effect of childhood rhGH therapy on bone compartments is affected by gender. In addition, indicators of the time of onset and aetiology of CO-GHD, but not additional pituitary hormone deficiencies, may have a larger influence on accrual of bone mass in these patients as those with congenital early

onset isolated GHD had lower bone mass that those with late-onset acquired GHD and MPHD. In terms of body composition, in consistent with some studies (229,233,236,241,245), CO-GHD patients had a significant lower LM for height and higher FM for height compared with controls. Our analysis also confirms that LM rather than FM had a stronger positive correlation with BMC and BMD at different sites TB/LS of either gender (381,524). However, once adjusted for age, height and weight, the data revealed that bone area was the strongest predictor of TB-BMC, followed by LM, in both CO-GHD and controls. Although several studies of children and adolescents have confirmed that both LM and FM are positively correlated with bone mass (525,526), there is a conflict over which has greater influence. In fact, the contribution of FM to bone mass has been inconsistent, with positive (527) and negative (528) relationships. Overall, our data confirmed that LM is an important factor in maximizing the chances of attaining the highest possible bone mass in patients with CO-GHD.

Considering the findings of chapter 4, our aims for chapter 5 were to study bone health and body composition of patients with CO-GHD at the time of initial evaluation prior to starting rhGH and retesting after withdrawal rhGH, and identifying any factors that may influence bone health in these subjects. In addition to DXA scans, we used pQCT scans for studying bone geometry and volumetric trabecular and cortical density separately. Muscle function and strength were assessed using jumping mechanography. Furthermore, we assessed bone profiles and mineralisation as well as biochemical markers of bone metabolism. Our results reveal that subjects with CO-GHD were not different in bone density and body composition parameters as measured by DXA and pQCT from those who had normal GH levels at either time points. Nevertheless, two main findings emerged from this study.

Firstly, though the sample size was small, our data clearly indicated declining muscle strength (max force) in naive GHD patients, which was proportional to their tibia muscle CSA. This finding is consistent with previously studies that reported low muscle strength in adolescents with CO-GHD after reaching final height (225,241) and in adulthood (423).

It is known that muscle strength is determined by the maximal force generated by fast twitch type II muscle fibres (high contractile force, but easy fatigability) (128). There is insufficient evidence that GH plays a role in the regulation of muscle fibre composition and induces a shift in muscle fibre from fibre type II (glycolytic fast-twitch fibres) to fibre type I (oxidative slow-twitch fibres) (128). However, it is unlikely that muscle weakness in GHD can be attributed to a shift in the distribution of fibre types, as muscle biopsies studies in adults with CO-GHD failed to identify any differences in fibre types compared with healthy controls (529). Therefore, it has been suggested that diminished muscle strength in GHD may arise from reduced muscle mass rather than from reduced contractile function (128). Therefore, regardless of the underlying mechanisms, it seems that reduction of muscle force is an early sign of alteration in musculoskeletal health in subjects with CO-GHD which is ultimately likely to play a key role in bone mass deficit in CO-GHD.

The second phenomenon to be considered from our analysis is the positive correlation between CTX levels and PTH levels time of diagnosis ($r = 0.46$, $p = 0.02$) and retesting ($r = 0.77$, $p = 0.02$). This relationship was supported by other observations of significantly higher CTX levels in naive GHD compared with normal, and a positive correlation between CTX levels and the duration of off rhGH in the retesting group. It is well known that PTH plays an important role in bone metabolism, and its fluctuations in concentration are important in determining its anabolic and catabolic bone effects (62,63). Experimental studies have suggested that low pulsatile secretion of PTH may have an important effect on bone formation and remodelling (anabolic effect), while continuous secretion of PTH may induce bone resorption and bone loss (catabolic effect) (530,531). GH may have a regulatory role in modulating PTH circadian rhythm and enhancing PTH anabolic effects, although the underlying mechanism remains unexplained (44,165). It has been previously described that untreated adults with GHD showed reduction in the sensitivity of PTH target organs (kidney and bone), with abnormalities in the PTH circadian pattern resulting in catabolic effects on bone metabolism without change in PTH concentration, and in maintaining calcium homeostasis (91,435). To our knowledge, there are no existing studies looking at PTH actions in children and adolescents with CO-GHD. Our data therefore suggest that serum PTH may be an important determinant of bone health in subjects with CO-GHD, and further studies are warranted.

In chapter 6 we aimed to investigate lipid profiles, glucose homeostasis and adipokine levels, and their relationship with bone metabolism markers and body composition in children and adolescents with CO-GHD at the time of initial evaluation and at retesting after they reached final height. Our data show no differences in lipid, adipokine and glucose homeostasis parameters in patients with CO-GHD and those with normal GH levels at both time points, and the majority of these parameters were within the normal range. In this study, however, we observed that the timing and duration of childhood treatment may influence the outcome of unfavorable lipid profile (total cholesterol) in adolescents with CO-GHD at final height. This relationship indicated that those who were older when they first started childhood rhGH and had a shorter duration of replacement before reaching their final height were more likely to have higher total cholesterol levels at final height after withdrawal of rhGH. There was a similar relationship between duration of childhood treatment but with HDL-cholesterol levels in adulthood reported previously by a study of the KIMS database (Pfizer International Metabolic Database): those who had a longer childhood duration of rhGH were likely to have higher HDL-cholesterol levels in adulthood (264). Although the causality remains unclear, we anticipated that, in keeping with the observation made from studies of adult with GHD (443,461), the favourable lipid profiles outcome of rhGH therapy in CO-GHD required an early commitment for long duration of rhGH therapy.

Our analysis in this study also revealed differences in the relationship between osteocalcin (bone formation marker) and leptin among our studied groups: they were positively correlated in the first-time assessment group but negatively in the retesting group. Although a number of studies have examined the relationship between leptin and bone metabolism in various cohorts, the results

remain conflicting because of the complexity of the leptin effect on bone with respect to age, gender and bone site and compartments (156). It appears that leptin affects bone metabolism either positively or negatively, and more research is needed to fully elucidate the role of leptin in the regulation of bone metabolism in subjects with CO-GHD.

The link between height, GHD and replacement and QoL has not been clarified to date. Therefore, the goal of chapter 7 was to evaluate QoL of children and adolescents with CO-GHD at the time of initial evaluation and at retesting after withdrawal of rhGH at final height. In this study, QoL was evaluated using the Short Form-36 (SF-36) generic health survey for the first-time assessment group and the Adult Growth Hormone Deficiency Assessment (AGHDA) disease-specific questionnaire for the retesting group. Our study demonstrated normal ranges of overall QoL aspects in children and adolescents with CO-GHD compared with subjects of the same stature but with normal GH levels. However, subscale analysis showed higher emotional wellbeing, but lower energy and vitality in naive GHD compared with normal. We were not able to explain whether this impairment is implicated directly in lack of energy due to GHD or partly caused by muscle weakness caused by GHD. Our retesting group also showed overall AGHDA scores within the range of the mean QoL AGHDA scores for the general population of the UK, with no significant differences between the medians scores of those who have persistent GHD and those with sufficient GH levels after retesting. However, an unexpected paradox finding was observed, with those who were GH-sufficient after retesting feeling significantly more tired and having poorer energy and vitality scores than those who had persistent GHD. This finding suggests that there are possible other unknown confounders aside from GH status which interfere with energy levels and tiredness in our cohort. The present finding also points to the importance of follow up and reassessment in those who had CO-GHD and were no longer GH deficient after retesting.

The overall results of our studies in this thesis revealed that muscle mass and strength are important contributors to bone health of subjects with CO-GHD. In addition, according to mechanostat theory (66), muscles mass and force cause mechanical loading on bones, and the subsequent bone response determine bone mass and strength. Therefore, enhancing muscle strength via exercise, for instance, is beneficial for bone mass accrual and bone strength. There is some evidence that suggests that jumping exercises may be a feasible and effective way to improve BMC when the subjects participate in training sessions at least three times a week over an average period of seven months (281,286,287). Therefore, chapter 8 aimed to investigate the feasibility and the effect of weight-bearing exercise (WBE) on bone health in subjects with CO-GHD. Although the study was based on a reasonable concept, unfortunately, because of a high dropout rate and low adherence, with such a small sample size, exploration of the effect of WBE was limited. Therefore, from our limited data, there was insufficient evidence to recommend the use of jumping exercise in the absence of rhGH in children and adults with CO-GHD. Further studies are necessary in different

circumstances to assess the optimum level and frequency of WBE on bone outcomes in children and adolescents with CO-GHD.

9.2 Conclusions

The overall conclusion of this thesis is that bone health in CO-GHD is not directly affected by GH status. Muscle mass and strength is the important contributor for optimal bone health in CO-GHD. PTH actions in CO-GHD could be another factor that impacts bone health, as indicated by increased CTX levels (bone resorption marker), and eventually results in bone loss. In the present study, there were no alterations observed in metabolic profiles and QoL of patients with CO-GHD between the initial evaluation and retesting. However, the early detection and commencement of rhGH treatment in CO-GHD is not only better for growth and bone health outcome, but also better for long-term metabolic and cardiovascular risks. Specific instruments are required to elucidate factors that modify the relationship between GH status and QoL in children and adolescents with CO-GHD. Whereas in this thesis, the effect of jumping exercises on the bone health and metabolic profiles was assessed, but for future research to be more informative, it may be useful to consider larger sample sizes to test programs, different settings and optimal doses that enhance bone health in children and adolescents with CO-GHD. Overall, careful follow-up is required to ensure optimal bone health in these children and adolescents with CO-GHD.

9.3 Limitations

There are several limitations, mainly related to the study populations and applied methodology, that were identified in this thesis.

The major drawback of our thesis is the limited sample size which limits the power to properly control for confounding variables and determine true associations. Furthermore, the wide age range including various pubertal stages might theoretically have weakened some of the relationships. The variety of aetiology and heterogeneity of our subjects with CO-GHD, and short stature of those with normal GH levels is another issue that was considered in our data. Although we applied several adjustment methods to DXA and pQCT bone assessment methods to avoid misinterpretation of the results, these techniques still do not account for other important parameters of bone strength, such as microarchitecture. Additional limitations drawn from the cross-sectional design of chapters 5, 6 and 7 make it difficult to determine causality. Another important issue is worth considering in chapter 7 that the QoL measures tools (SF-36- AGHDA) may lack sensitivity to detect the differences between our studied groups. No existing measures have not been developed for or validated within the population of children and adolescents with CO-GHD. For our longitudinal study (chapter 8), we believed that it would be relatively easy to recruit a larger number of patients. However, the study did not achieve the requisite 60 subjects; 36 subjects were recruited for the study and only 19 subjects completed the study. Poor adherence to the assigned

exercise program is a critical challenge in our study. Loss of motivation to adhere to an exercise programme is a remarkable cause of poor compliance.
A final limitation is that we did not measure the patients' habitual physical activity objectively. Although in our initial proposal we intended to use accelerometers to measure habitual physical activity, owing to technical problems and the loss of devices, the high costs, and limited availability of such devices made us unable to continue to provide accelerometers.

9.4 Future Directions

In this thesis we studied the bone health and body composition in subjects with CO-GHD and the underlying mechanism of deterioration of bone health was proposed. These encouraging results lead us to feel that there is some promise in this area for further research. Many of the research questions which stem from the present thesis would be ideally investigated in further studies.

1- What is the optimal management of CO-GHD during transition? Further studies are needed on the current practice of assessment and management of CO-GHD and to investigate the long term follow up of patients with reconfirmed GHD, whether or not they opt to continue on adult rhGH replacement in adulthood.

2- What is the relationship between bone health measurements and risk of fracture in CO-GHD? A prospective study on patients with newly diagnosed CO-GHD would be optimal to evaluate bone health development, using more advanced non-invasive imaging tools such as micro-MRI, with special emphasis on evaluation the functional muscle-bone relationship in clinical siting may be more useful in these patients. The mechanisms of PTH action and its interaction with GH warrant further investigation in subjects with CO-GHD. Additionally, long-term follow up of the patients until late adolescence is required to evaluate their peak bone mass, a major determinant of osteoporosis later in life.

3- How does CO-GHD impact on individual quality of life during childhood and adolescence, and does rhGH treatment improve QoL of these subjects? Further longitudinal studies using more specific validation instruments are needed to assess QoL in children and adolescents with GHD before, during and after initiating rhGH treatment.

4- What is the optimal and feasible physical activity programme for bone health of subjects with CO-GHD? Further research is needed to test the efficacy, effectiveness, and feasibility of implication of exercises regimen in different settings that encompasses both bone and metabolic health of subjects with CO-GHD. It must take into account, however, any exercise regimen must be considered alongside rhGH, and more attention is required to identify factors that enhance long-term adherence, motivation, and interest.

Appendices

10.1 Appendix A: Proforma Form of an audit of the management of childhood-onset growth hormone deficiency during young adulthood in Scotland

Hospital ID: _____	Sex: ☐ Male ☐ Female	Date of Birth: _____	Parents height Mother: _____ Father: _____ Mid parental height_____	
Height prior start GH	Measurement date	Final Height	Weight at final height	Measurement date
Cms:_____	_____	Cms:_____	Kgs:_____	_____
Puberty induction □No □ Yes	Induction date _____			
Other pituitary deficiencies □ Thyroid □ Cortisol □ Sex steroids □ DDAVP				

Medical history [clinical diagnosis and medications]

Diagnosis /medications	Date of onset

Growth hormone stimulation test

Reasons for testing/underlying causes for GHD

Short Stature assessment ☐

CNS tumours ☐

Cranial radiation ☐

Others ☐

Specify :_____

Diagnostic GH stimulation test date_____

Type of test ☐ ITT

☐ Arginine

☐ Other-state_____

GH peak response at diagnosis ☐ IGF1 level ☐

Date of start GH therapy_____

Date of stop GH therapy_____

referral for re-evaluation ☐No ☐ Yes

Date of referral _____

Date of re-testing_____

Type of test

☐ ITT

☐ Arginine

☐ Other-state_____

GH peak response at retesting ☐

IGF1 level at retesting ☐

Imaging

Pituitary MRI: □No □ Yes **Date :**_____

Pituitary MRI report: _____

DXA: □No □ Yes **Date :**_____

Reasons for DXA:_____

DXA report: _____

Clinical decision

Stop GH □No □ Yes

Adult GH □No □ Yes

Patient decision- continue with adult GH □No □ Yes

Additional information

10.2 Appendix B: SF-36(tm) Health Survey

SF-36(tm) Health Survey

Instructions for completing the questionnaire: Please answer every question. Some questions may look like others, but each one is different. Please take the time to read and answer each question carefully by filling in the bubble that best represents your response.

Patient Name: _____

SSN#: _____ Date: _____

Person heling to complete this form: _____

1. In general, would you say your health is:

   - ❑ Excellent
   - ❑ Very good
   - ❑ Good
   - ❑ Fair
   - ❑ Poor

2. Compared to one year ago, how would you rate your health in general now?

   - ❑ Much better now than a year ago
   - ❑ Somewhat better now than a year ago
   - ❑ About the same as one year ago
   - ❑ Somewhat worse now than one year ago
   - ❑ Much worse now than one year ago

3. The following items are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

   a. Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports.
      - ❑ Yes, limited a lot.
      - ❑ Yes, limited a little.
      - ❑ No, not limited at all.

   b. Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf?
      - ❑ Yes, limited a lot.
      - ❑ Yes, limited a little.
      - ❑ No, not limited at all.

   c. Lifting or carrying groceries.
      - ❑ Yes, limited a lot.
      - ❑ Yes, limited a little.
      - ❑ No, not limited at all.

   d. Climbing several flights of stairs.
      - ❑ Yes, limited a lot.
      - ❑ Yes, limited a little.
      - ❑ No, not limited at all.

   e. Climbing one flight of stairs.
      - ❑ Yes, limited a lot.
      - ❑ Yes, limited a little.
      - ❑ No, not limited at all.

   f. Bending, kneeling or stooping.
      - ❑ Yes, limited a lot.
      - ❑ Yes, limited a little.
      - ❑ No, not limited at all.

g. Walking more than one mile.
- ❑ Yes, limited a lot.
- ❑ Yes, limited a little.
- ❑ No, not limited at all.

h. Walking several blocks.
- ❑ Yes, limited a lot.
- ❑ Yes, limited a little.
- ❑ No, not limited at all.

i. Walking one block.
- ❑ Yes, limited a lot.
- ❑ Yes, limited a little.
- ❑ No, not limited at all.

j. Bathing or dressing yourself.
- ❑ Yes, limited a lot.
- ❑ Yes, limited a little.
- ❑ No, not limited at all.

4. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

a. Cut down the amount of time you spent on work or other activities?
☐ Yes ☐ No

b. Accomplished less than you would like?
☐ Yes ☐ No

c. Were limited in the kind of work or other activities
☐ Yes ☐ No

d. Had difficulty performing the work or other activities (for example, it took extra time)
☐ Yes ☐ No

5. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

a. Cut down the amount of time you spent on work or other activities?
☐ Yes ☐ No

b. Accomplished less than you would like
☐ Yes ☐ No

c. Didn't do work or other activities as carefully as usual
☐ Yes ☐ No

6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?
- ❑ Not at all
- ❑ Slightly
- ❑ Moderately
- ❑ Quite a bit
- ❑ Extremely

7. How much bodily pain have you had during the past 4 weeks?
- ❑ Not at all
- ❑ Slightly
- ❑ Moderately
- ❑ Quite a bit
- ❑ Extremely

8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

- ❑ Not at all
- ❑ Slightly
- ❑ Moderately
- ❑ Quite a bit
- ❑ Extremely

9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks.

a. did you feel full of pep?

- ❑ All of the time
- ❑ Most of the time
- ❑ A good bit of the time
- ❑ Some of the time
- ❑ A little of the time
- ❑ None of the time

b. have you been a very nervous person?

- ❑ All of the time
- ❑ Most of the time
- ❑ A good bit of the time
- ❑ Some of the time
- ❑ A little of the time
- ❑ None of the time

c. have you felt so down in the dumps nothing could cheer you up?

- ❑ All of the time
- ❑ Most of the time
- ❑ A good bit of the time
- ❑ Some of the time
- ❑ A little of the time
- ❑ None of the time

d. have you felt calm and peaceful?

- ❑ All of the time
- ❑ Most of the time
- ❑ A good bit of the time
- ❑ Some of the time
- ❑ A little of the time
- ❑ None of the time

e. did you have a lot of energy?

- ❑ All of the time
- ❑ Most of the time
- ❑ A good bit of the time
- ❑ Some of the time
- ❑ A little of the time
- ❑ None of the time

f. have you felt downhearted and blue?

- ❑ All of the time
- ❑ Most of the time
- ❑ A good bit of the time
- ❑ Some of the time
- ❑ A little of the time
- ❑ None of the time

g. did you feel worn out?

- ❑ All of the time
- ❑ Most of the time
- ❑ A good bit of the time
- ❑ Some of the time
- ❑ A little of the time
- ❑ None of the time

h. have you been a happy person?

- ❑ All of the time
- ❑ Most of the time
- ❑ A good bit of the time
- ❑ Some of the time
- ❑ A little of the time
- ❑ None of the time

i. did you feel tired?

- ❑ All of the time
- ❑ Most of the time
- ❑ A good bit of the time
- ❑ Some of the time
- ❑ A little of the time
- ❑ None of the time

10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?

- ❑ All of the time
- ❑ Most of the time
- ❑ Some of the time
- ❑ A little of the time
- ❑ None of the time

11. How TRUE or FALSE is each of the following statements for you?

a. I seem to get sick a little easier than other people

- ❑ Definitely true
- ❑ Mostly true
- ❑ Don't know
- ❑ Mostly false
- ❑ Definitely false

b. I am as healthy as anybody I know

- ❑ Definitely true
- ❑ Mostly true
- ❑ Don't know
- ❑ Mostly false
- ❑ Definitely false

c. I expect my health to get worse

- ❑ Definitely true
- ❑ Mostly true
- ❑ Don't know
- ❑ Mostly false
- ❑ Definitely false

d. My health is excellent

- ❑ Definitely true
- ❑ Mostly true
- ❑ Don't know
- ❑ Mostly false
- ❑ Definitely false

10.3 Appendix C: The Quality of Life-Assessment of Growth Hormone Deficiency in Adults Questionnaire

LISTED BELOW ARE SOME STATEMENTS that people may make about themselves.

Read the list carefully and put a tick in the box marked YES if the statement applies to you.

Tick the box marked NO if it does not apply to you.

Please answer every item. If you are not sure whether to answer YES or NO, tick whichever answer you think is most true in general.

	YES	NO
I have to struggle to finish jobs	☐	☐
I feel a strong need to sleep during the day	☐	☐
I often feel lonely even when I am with other people	☐	☐
I have to read things several times before they sink in	☐	☐

	YES	NO
It is difficult for me to make friends	☐	☐
It takes a lot of effort for me to do simple tasks	☐	☐
I have difficulty controlling my emotions	☐	☐
I often lose track of what I want to say	☐	☐

	YES	NO
I lack confidence	☐	☐
I have to push myself to do things	☐	☐
I often feel very tense	☐	☐

QoL-AGHDA English
2000-02-17
OR 7064-01

	YES	NO
I feel as if I let people down	☐	☐
I find it hard to mix with people	☐	☐
I feel worn out even when I've not done anything	☐	☐

	YES	NO
There are times when I feel very low	☐	☐
I avoid responsibilities if possible	☐	☐
I avoid mixing with people I don´t know well	☐	☐

	YES	NO
I feel as if I m a burden to people	☐	☐
I often forget what people have said to me	☐	☐
I find it difficult to plan ahead	☐	☐
I am easily irritated by other people	☐	☐

	YES	NO
I often feel too tired to do the things I ought to do	☐	☐
I have to force myself to do all the things that need doing	☐	☐
I often have to force myself to stay awake	☐	☐
My memory lets me down	☐	☐

Now please go back to the first question and make sure that you have answered "YES" or "NO" to every question, on all two pages of the questionnaire. Thank you for your help.

QoL-AGHDA English
2000-02-17
OR 7064-01

10.4 Appendix D: Exercise Regimen

Exercise Regimen

The exercise program can be performed just about anywhere with little effort. Always start the exercise program with the minimum amount of jumps, and then increase the number of jumps when it becomes easier for your child.

Please, make sure your child is supervised by an adult to record him performing the exercise using provided video camera

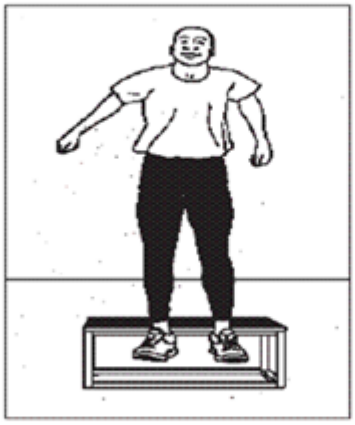
Actions:

1- Stand on

Stand on the platform standing up with feet together and hands down by your side

2- Jumping

Push up from the platform, jumping up in the air as high as he/she can



3- Landing

Land softly on both feet with knees slightly bent onto a hard flat surface

4- Repeat 10-25 jumps

5- Do these exercise three days /week whenever you want

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